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THE ROLE OF NEW ULTRASOUND MODALITIES TO REFINE THE DIAGNOSTIC WORKUP ON WOMEN WITH CERVICAL CARCINOMA

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The role of new ultrasound modalities to refine the diagnostic workup on women with cervical carcinoma

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To my parents
Mamma og pabbi þessi bók er tileinkuð ykkur
Menntun er máttur

Abstract

Background: Cervical cancer is the fourth most common female cancer in the world and has traditionally been clinically staged not including imaging. Despite that, clinicians depend on diagnostic imaging in the management of patients to triage them to treatment. Ultrasonography (US) is the most widely used diagnostic imaging technique by gynecologists worldwide and is equally accurate as Magnetic resonance imaging (MRI) for local assessment of cervical cancer in the pelvis in low volume disease, whereas both methods lack accuracy for detection of lymph node metastasis. New ultrasound modalities such as three-dimensional ultrasonography (3D US), contrast-enhanced ultrasonography (CEUS) and strain elastography (SE) have shown promising results in the assessment of tumours, but the clinical value in cervical cancer patients is uncertain. The aims of this thesis were to explore the clinical value of 3D US, CEUS and SE and to assess the inter-rater agreement of conventional US and MRI in the assessment of patients with cervical cancer.

Methods: Study I was a multicenter study including 104 women with surgically resectable cervical cancer from 5 European institutions. **Studies II-IV** were based on a single center cohort of 93 women with all stages of cervical cancer, from Karolinska University Hospital. In **study I** subjective evaluation was compared to objective measurements of 2D and 3D US parameters for prediction of deep stromal and parametrial invasion as well as lymph node metastases. In **study II** semi-quantitative parameters and filling pattern of CEUS were compared in 49 patients with cervical cancer and 21 healthy controls. The features of SE in 30 patients with all stages of cervical cancer were explored in **study III.** In **study IV** the inter-rater agreement of raters with varying experience on US and MRI were compared in off-line settings for the assessment of cervical tumours in a cohort of 60 patients with all stages of cervical cancer.

Results: Subjective assessment with 2D US had high accuracy to detect deep stromal and parametrial invasion (sensitivity 91% and 100%; specificity 97% and 95% respectively) but not to predict lymph node metastases. Tumour size measured by 2D and volume measured by 3D US were accurate to predict deep stromal invasion (AUC 0.83 and 0.85) but outperformed by subjective assessment. 3D vascular indices (VI, VFI, FI) had no value to predict deep stromal invasion or lymph node metastases. A focal CEUS pattern had a higher specificity 91% versus 73% (p=0.62) and a similar sensitivity 80% versus 85% (p=1.00) compared to subjective assessment using conventional US for tumour detection. The semi-quantitative CEUS parameter area under the time intensity curve had high accuracy to separate tumour lesions from healthy stroma (AUC 0.923, p<0.001). Size measures of early stage tumours were accurate with SE (mean difference -0.11 mm, p=0.66) with no bias found. An elasticity score of 4-5 was found in 45% (9/20) with early stage and 80% (8/10) with advanced disease where SE was even useful in 7/10 (70%) of cases to delineate tumour borders. The inter-rater agreement for tumour visualization, 1/3 deep stromal and parametrial invasion was moderate for US raters (0.4-0.6) and moderate-good (0.4-0.8) for MRI raters irrespective of experience.

Conclusion: Subjective 2D US assessment is accurate for detecting deep stromal and parametrial invasion where it outperforms objective measurements with 2D and 3D vascular indices. A focal contrast pattern with CEUS has high specificity and high accuracy for tumour detection. SE is accurate to measure tumour size and may improve delineation of advanced tumours. In off-line settings, inter-rater agreement is moderate for US raters and moderate-good for MRI raters irrespective of experience.

List of scientific papers

I. Pálsdóttir K, Fischerova D, Franchi D, Testa A, Di Legge A, Epstein E

Preoperative prediction of lymph node metastasis and deep stromal invasion in women with invasive cervical cancer: prospective multicenter study using 2D and 3D ultrasound
Ultrasound Obstet Gynecol 2015; 45:470-475

II. Pálsdóttir K, Epstein E

A pilot study on diagnostic performance of contrast-enhanced ultrasonography for detection of early cervical cancer
Ultrasound in Med.& Biol 2018; 44 (8): 1664-1671

III. Pálsdóttir K, Mogensen O, Epstein E

Ultrasound Strain elastography features in patients with early and advanced stages of cervical cancer
Manuscript

IV. Fridsten S and Pálsdóttir K, Blomqvist L, Hasselrot K, Alagic Z, Sundin A, Epstein E

The inter-observer agreement using MRI and Ultrasound to assess tumour extension in patients with all stages of cervical cancer – impact of reader experience on reproducibility and accuracy
Manuscript

Contents

List of abbreviations

ACRIN	American college of radiology imaging network
AIS	Adenocarcinoma in situ
AIU	Arbitrary Intensity Unit
ARH	Abdominal radical hysterectomy
AUC	Area under the curve
CEUS	Contrast enhanced ultrasonography
CI	Confidence Interval
CIN	Cervical intraepithelial neoplasia
CRF	Case report form
CT	Computed Tomography
DFS	Disease free survival
EBRT	External beam radiation therapy
EFSUMB	European federation of societies for ultrasound in medicine and biology
FDG PET	Fluorodeoxyglucose Positron Emission Tomography
FI	Flow index
FIGO	The International Federation of Obstetrics and Gynecology
FN	False negative
FP	False positive
GOG	Gynecological oncology group
HIV	Human immunodeficiency virus
HPV	Human papilloma virus
HRHPV	High risk Human papilloma virus
HR	Hazard ratio
ICG	Indo cyanine green
IOTA	The International Ovarian Analysis group
LACC	Laparoscopic approach in cervical cancer
LVSI	Lymph vascular space invasion
MHz	Mega Hertz
MIS	Minimally invasive surgery
MRI	Magnetic resonance imaging
NACT	Neoadjuvant Chemotherapy
NPV	Negative predictive value
OS	Overall survival
PACS	Picture Archiving and Communication System
PAP	Papanicolaou
PCF	Pericervical fascia
PD	Power Doppler
PET-CT	Positron emission tomography – computed tomography
PPV	Positive predictive value
PRICE	Prospective Imaging of Cervical cancer and neoadjuvant treatment - trial
PRF	Pulse repetition frequency
RALS	Robot assisted laparoscopic surgery
RCT	Radiation chemotherapy
ROI	Region of interest
RRH	Robot assisted radical hysterectomy
RTOG	Radiation Therapy Oncology Group
SD	Standard deviation
SE	Ultrasound strain elastography
SHAPE trial	Radical versus simple hysterectomy and pelvic node dissection in patients with low risk early stage cervical cancer
SNB	Sentinel node biopsy
SPSS	Statistical Package for Social Sciences
TIC	Time intensity curve
TMMR	Total mesometrial resection
TN	True negative
TP	True positive
TRS	Transrectal ultrasonography
TVS	Transvaginal ultrasonography
US	Ultrasonography
VFI	Vascularization-flow index
VI	Vascularization index
2D	Two dimensional
3D-PD	Three-dimensional Power Doppler
3D US	Three dimensional ultrasonography

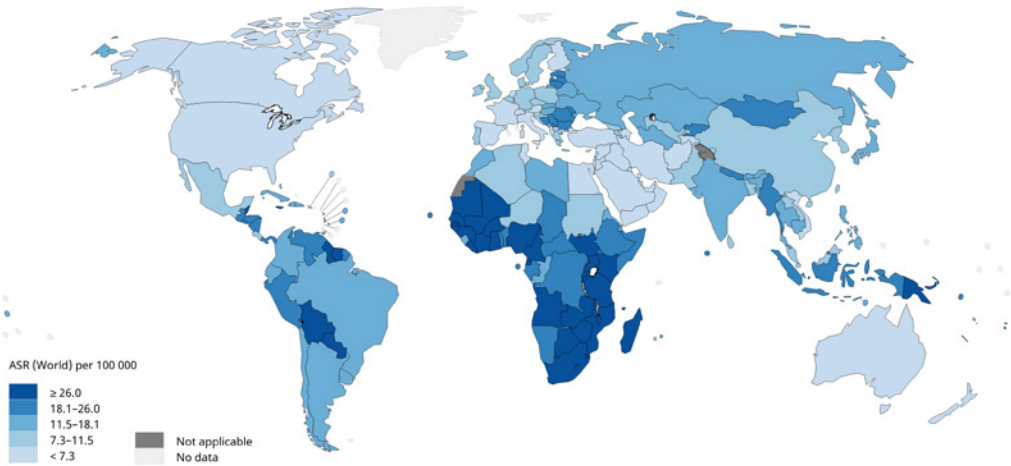


Figure 1. The estimated age-standardized incidence rates for cervical cancer globally in 2018. Data source: GLOBOCAN 2018, Graph production: IARC (http://gco.iarc.fr/today) World Health Organization.

1 Introduction

Ultrasonography (US) is the most widely used diagnostic modality in gynecology worldwide. Studies have verified a high accuracy of the method for evaluation of patients with low volume cervical cancer (1) while new modalities such as three-dimensional ultrasonography (3D US), contrast enhanced ultrasound (CEUS) and strain elastography (SE) are less studied.

In a global perspective cervical cancer is a major burden as the fourth most common cancer among females (2) (Figure 1). As a result of successful prevention with nationwide screening programs and since recently vaccination, cervical cancer is now relatively uncommon in the Nordic countries (3). Due to lacking resources where cervical cancer has the highest incidence, the disease has been staged clinically according to The International Federation of Obstetrics and Gynecology (FIGO). Studies have reported the clinical system as insufficient, both in terms of low accuracy and by ignoring disease status of the pelvic lymph nodes, an important prognostic factor. Recently a new staging system was introduced (4) allowing findings from imaging such as Magnetic Resonance Imaging (MRI) and US to determine disease stage. MRI has since 15 years been included in the management of patients with cervical cancer in high-income countries both for diagnostic workup as well as treatment planning. The limitations are in the diagnostic workup of small tumours < 10 mm, the low sensitivity for detection of lymph node metastasis and restricted access in many areas in the world (5). As an inexpensive, widely available and accurate method, ultrasonography is an important alternative and complement to MRI even if the limitations are the same regarding assessment of lymph nodes. To be able to further refine the diagnostic accuracy for local tumour assessment and lymph node status new methods are required.

This thesis focuses on the value of new ultrasound techniques as a complement to conventional US in the assessment of patients with cervical cancer as well as the reliability of US in comparison to MRI in this patient group.

2 Background

2.1 ANATOMY

The uterine cervix is the most caudal part of the female uterus, marking the borders between uterus and the vagina. It is derived from the Müllerian ducts bilateral in the embryo that by fusion in the midline form the fallopian tubes, uterus, the uterine cervix, the upper third of the vagina as well as the tissue surrounding these organs (6). The uterine cervix is composed of 90% fibrotic stromal tissue and 10% smooth muscle surrounding the fibrous stroma more superficially, connecting the cervix with the vaginal wall. The connective tissue lateral to the uterine cervix, including mainly lymphatic tissue and vessels to the uterus and vagina is in surgical terms called the lateral parametrium but anatomically the cardinal ligament. The dorsal part connecting the uterine cervix to the sacrum are the bilateral uterosacral ligaments, that form the posterior parametrium and mark the lateral borders on both sides for the rectovaginal space (7) (Figure 2). In front of the cervix lies the urinary bladder in close adjunction covering the isthmus of the uterus and the anterior cervix. Where the ureters enter the bladder anterolaterally on both sides is called the ventral or anterior parametrium. In collective terms all the the tissue surrounding the cervix is named pericervical tissue.

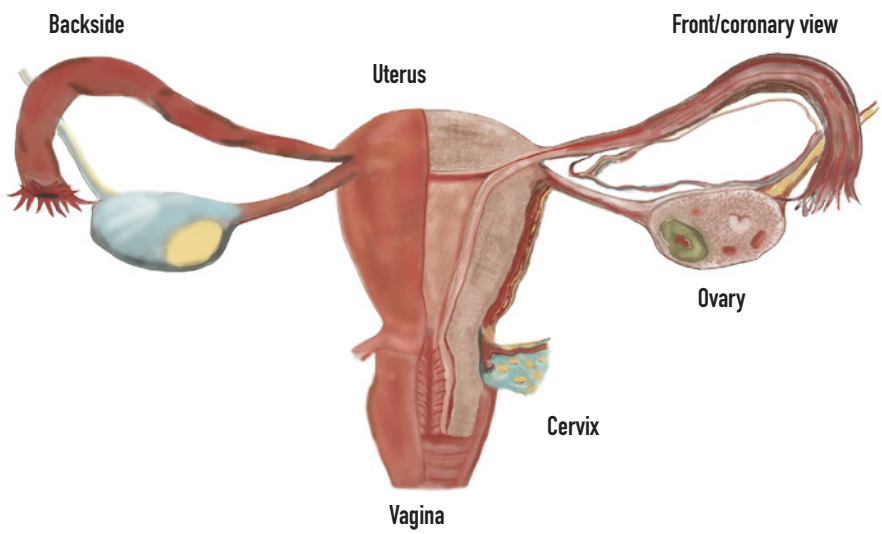


Figure 2a. Anatomy of the inner female genitalia. Figure: Caroline Castoriano Gade

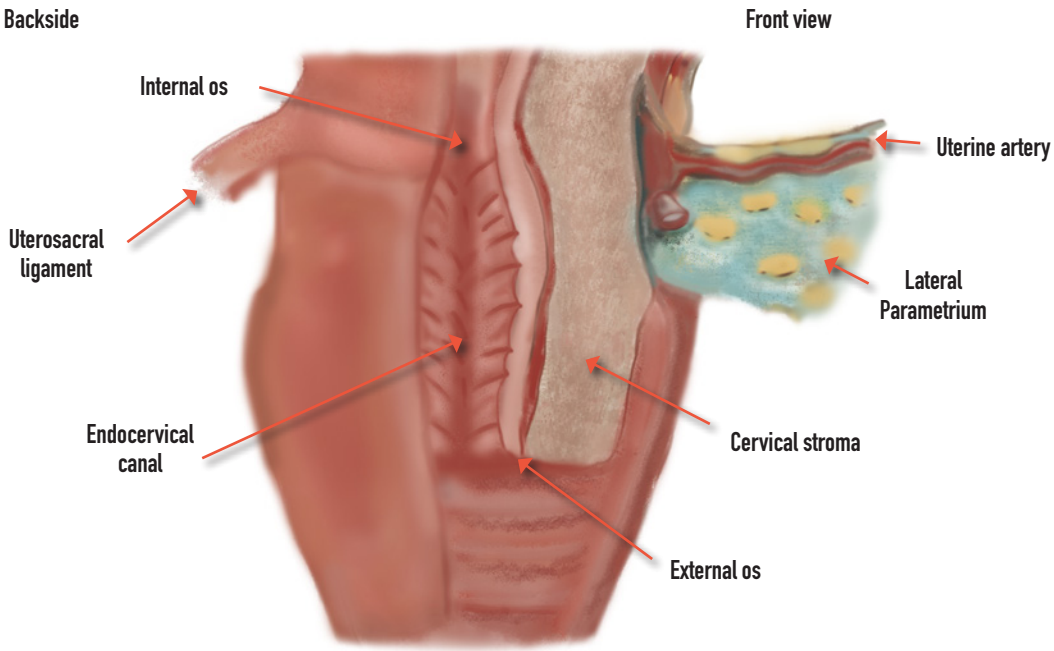


Figure 2b. Uterine cervix with surrounding structures. Figure: Caroline Castoriano Gade.

The vaginal part of the uterine cervix is covered by the same squamous epithelium as rest of the vagina. The vaginal wall and cervix meet circumferentially about 1,5 or 2 cm from the most caudal part of the cervix forming the vaginal fornices. The endocervical canal leading to the uterine cavity is covered by columnar epithelium. These two different layers meet at the junctional zone at the external os which is variably exposed in relation to hormonal changes and age. At this fusion site the epithelium is most vulnerable and for that reason the majority of cervical cancer lesion grow from there. The most usual growth pattern for cervical tumours is through the cervical stroma and into the parametrium laterally, ventrally or dorsally furthermore to lymph nodes in the pelvic or paraaortic region. In the more exofytic growing lesions the tumours tend to disseminate onto the vaginal wall and fornices.

2.2 EPIDEMIOLOGY

Cervical cancer is the fourth most common malignancy among females worldwide, with the largest proportion of women affected in low and middle income countries (2) (Figure 1). In Sweden and other western countries it is less common as the third most common gynecological malignancy after endometrial cancer and ovarian

cancer (8). However, the yearly incidence has increased since 2014 with a peak of 575 new cases in the year 2015. Standardized for age the yearly incidence is from 6.6-8.8/100.000 which is comparable to rest of the Nordic countries (9) (Figure 3). The incidence is highest in women in the forties when 1/3 of all cases are diagnosed and in women around 70 years, with a median age 48 years at diagnosis. Prognosis is strongly related to the dissemination of disease at the time of diagnosis with five years overall survival (OS) > 90% for patients diagnosed in stage I with negative pelvic lymph nodes (10).

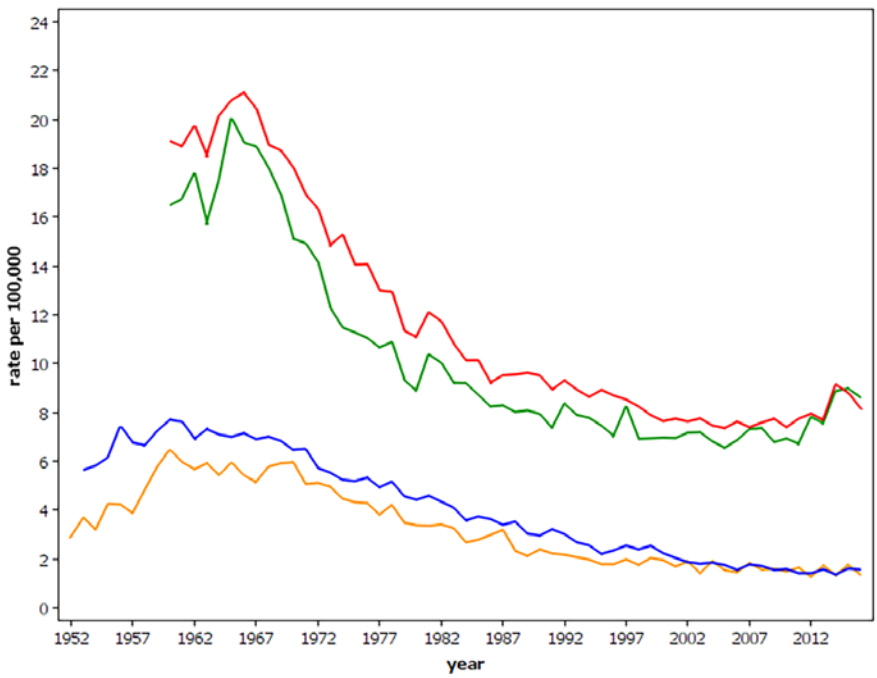


Figure 3. Age standardized incidence (red line) and mortality (blue) of cervical cancer in the Nordic countries and age standardized incidence (green) and mortality (yellow) in Sweden. Statistics and figure from NORDCAN(3).

2.3 RISK FACTORS AND PREVENTION

2.3.1 HPV infection

The precursor of the disease; cervical intraepithelial neoplasia (CIN, dysplasia) is without symptoms and is caused by a sexually transmitted infection by human papilloma virus (HPV). There are hundreds of different HPV strains where some have been strongly associated with the risk of developing cervical cancer (11). Those together are called high-risk strains or HRHPV. The most common high risk types are

16 and 18 that together are found in about 70% of all cervical cancers (11). Other strains include 31, 33, 45, 58 and many more as at least fifteen different high-risk types have been identified (12). HRHPV is found in all cervix cancer lesions (13) and is a necessary but not a sufficient trigger alone to cause the disease. Over 90% of women who get infected do not develop cervical cancer but heal from the infection spontaneously within two years (14). However in those with persistent infection especially type 16 the risk of developing high-grade dysplasia or cancer is increased with up to 22% (15).

2.3.2 Other risk factors

It is not fully understood why some women develop chronic HPV infection and thereby have increased risk of developing cancer. Some co-factors associated with increased risk for cervical cancer have been identified such as: long term use of oral-contraceptive especially in combination with smoking, high number of live births, smoking and increasing number of sex partners (16-18). Immunologically deprived individuals such as HIV positive or transplanted women have a higher risk of persistent HPV infection, high grade CIN and invasive cervical cancer due to impaired cell-mediated immunity (19, 20).

2.3.3 Prevention

Since the 1960s there is a screening program that through nationwide coverage reaches to women in all regions in Sweden (21). Through the years the method has evolved from Papanicolaou (Pap) smear on microscopic glass into today's liquid based cytology tests with possibilities to simultaneously test for high-risk HPV types. According to the newly updated national guidelines for cervical cancer prevention by the National board of Health and Welfare in Sweden (Socialstyrelsen), the recommended age of women for screening is 23–64 years with liquid-based cytology, including HPV testing for women older than 30 years with recommended interval for testing 3 years for age group 23-49 and seven years for women 50 years and older (22). The inclusion of HPV testing in to the screening program is based on the results from 4 large European randomized controlled trials showing a 60% higher protection against cervical carcinoma with HPV screening compared to traditional cytology as the HPV testing targets the high risk population (23). In cases of high grade CIN or persistent low-grade dysplasia with HRHPV the recommended treatment is destruction of the junction zone / epithelium of the cervix usually by cone biopsy of the uterine cervix. In that way most infections are effectively cured with minimized risk for cancer later in life (24).

2.3.4 Vaccination

Introduction of bivalent, quadrivalent and the new ninevalent HPV vaccine is expected to decrease the incidence of cervical cancer. All the vaccines are effective against the most common types 16 and 18, the quadrivalent type further protects against type 6 and 11 just as the ninevalent vaccine that in addition protects against types 31, 33, 45, 52 and 58 (25-27). Papers have already emerged reporting long-term effectiveness, decreased incidence of CIN, Adenocarcinoma in situ (AIS) and persistent infection by high-risk subtypes of HPV mostly 16 and 18 in vaccinated populations (28-30). A nationwide vaccination program for girls 10-12 years old started 2012 in Sweden, aiming for vaccination before sexual debut. This is expected to reverse the negative trend seen during the last years with increasing incidence of cervical cancer although it will take at least a decade before the effect becomes prominent.

2.4 HISTOLOGY

The most common histology type is squamous cell carcinoma accounting for 75-80% of cases (31, 32). Adenocarcinoma is diagnosed in about 20% of cases as the second most common type. Other types like mixed adenosquamous type, clear cell carcinoma and neuroendocrine small cell cancer are less common but more aggressive with worse prognosis, especially the neuroendocrine small cell type that often presents with distant metastases at the time of diagnosis (33). In the last decades the incidence of adenocarcinoma has been increasing at the same time as the incidence of squamous cell carcinoma has decreased (8). This indicates lacking efficacy of the screening program finding the precursors of adenocarcinoma.

2.5 CLINICAL SIGNS AND STAGING

With an established invasive lesion of cervical cancer, the main symptoms are: vaginal bleeding after intercourse, pelvic pain and abnormal vaginal discharge. Some women with cervical cancer present late and without symptoms, especially with adenocarcinoma growing in the cervical canal where the lesion is not always visualized by vaginal examination (Figure 4). Usually the tumour is seen macroscopically where further histology is verified by a tissue biopsy. Other tumours are diagnosed at early stages by cone biopsy, performed as a result of dysplasia found with routine screening. In Swedish population, approximately 30% of all cervical cancer is found by screening especially in younger patients (34-36).

Traditionally cervical tumours have been staged according to the International Federation of Obstetrics and Gynecology (FIGO) clinical staging criteria, (Table 1)

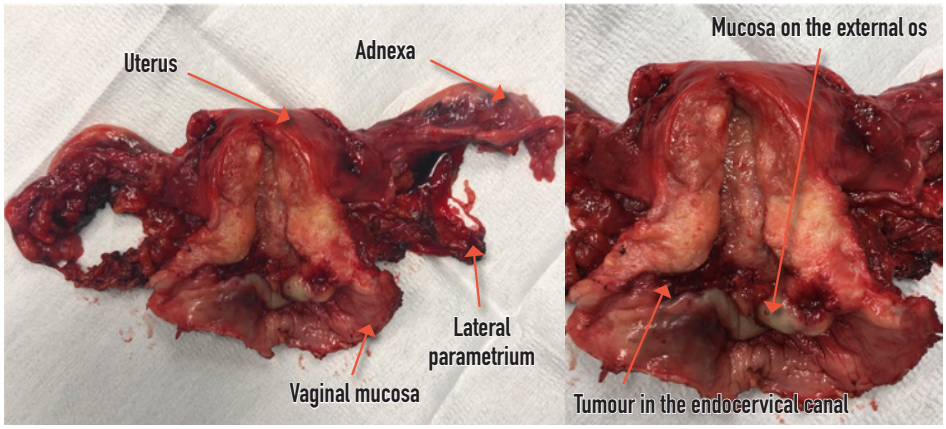


Figure 4. A specimen from radical surgery, a) Showing from above down: Uterus with adnexa, lateral parametrium and vaginal mucosa. B) uterus and uterine cervix; Arrows pointing at tumour in the endocervical cancal, covered by mucosa on the external cervical os. The tumour was difficult to visualise macroscopically by gynecological examination.

(37). It includes gynecological examination under anesthesia with palpation of the pelvis, cystoscopy, rectoscopy and if needed X-ray of the lungs. No obligatory imaging was included but according to the revised FIGO criteria from 2009, usage of imaging modalities such as magnetic resonance imaging (MRI) was encouraged (37). The reason for excluding advanced imaging has been the lack of available imaging modalities in countries with limited resources where cervical cancer is common. Studies have found the clinical classification system underestimating the disease stage both the early stage 15-30% and the more advanced stages up to 40% at stage IIIB as compared to surgical staging (38-40) as well as not taking into consideration the metastatic status of lymph nodes. A moderate inter-observer agreement for clinical staging in the hands of experienced examiners has been reported (41). For this reason and because of the clinical significance of lymph node metastases a new staging system was introduced by FIGO 2018 that includes imaging and pathology in addition to clinical findings to decide disease stage, thereby incorporating a new stage IIIC (lymph node metastases) into the staging system (Table 1). Furthermore lateral extension of the microscopic lesions IA has been eliminated and by adding IB3 for tumours > 4 cm the former stage IB1 becomes IB2. It is recommended that when in doubt the lower staging should be assigned (4). For clarification in this thesis, FIGO classification system from 2009 is applied unless otherwise specified.

Table 1. FIGO staging for cervical carcinoma combined 2009 and 2018 where red letters mark changes to the system applied 2018. Adapted from (4, 42)

2018	2009	Definition
I	I	The carcinoma is strictly confined to the cervix (extension to the uterine corpus should be disregarded)
	IA	Invasive cancer identified only microscopically (All gross lesions even with superficial invasion are Stage IB cancers) Invasion is limited to measured stromal invasion with a maximum depth of 5 mm and no wider than 7 mm.
IA		For FIGO 2018 only maximum depth of invasion ≤ 5 mm
IA1	IA1	Measured invasion of stroma ≤ 3 mm in depth (and ≤ 7 mm width)
IA2	IA2	Measured invasion of stroma > 3mm and < 5 mm in depth (and ≤ 7 mm width)
	IB	Clinical lesions no greater than 4 cm in size
IB		Invasive carcinoma with measured deepest invasion ≥ 5 mm (greater than IA)
	IB1	Clinical lesions no greater than 4 cm in size
IB1		Invasive carcinoma ≥ 5 mm depth of stromal invasion, and < 2 cm in greatest dimension
	IB2	Clinical lesions > 4 cm in size
IB2		Invasive carcinoma ≥2 cm and <4 cm in greatest dimension
IB3		Invasive carcinoma ≥4 cm in greatest dimension
II	II	The carcinoma extends beyond the uterus, but has not extended onto the pelvic wall or the lower third of vagina
IIA	IIA	Involvement of up to the upper 2/3 of the vagina. No obvious parametrial involvement
IIA1	IIA1	Invasive carcinoma <4 cm in greatest dimension
IIA2	IIA2	Invasive carcinoma ≥4 cm in greatest dimension
IIB	IIB	(Obvious) parametrial involvement but not onto the pelvic sidewall
III	III	The carcinoma has extended onto the pelvic sidewall. On rectal examination there is no cancer free space between the tumour and pelvic sidewall. The tumour involves the lower third of the vagina. All cases of hydronephrosis/nonfunctioning kidney should be included unless they are known to be due to other causes
IIIA	IIIA	Involvement of the lower vagina but no extension onto the pelvic sidewall
IIIB	IIIB	Extension onto the pelvic sidewall, or hydronephrosis/nonfunctioning kidney (unless known due to other cause)
IIIC		Involvement of pelvic and/or paraaortic lymph nodes, irrespective of tumour size and extent (with r=radiology and p=pathology notations)
IV	IV	The carcinoma has extended beyond the true pelvis or has clinically involved the mucosa of the bladder and/or rectum. (Biopsy proven)
IVA	IVA	Spread to adjacent pelvic organs
IVB	IVB	Spread to distant organs

2.6 TREATMENT

2.6.1 Radical surgery

In the year 1951 Dr. Meigs published data on 100 patients with cervical cancer stage I and II. He described a modified radical hysterectomy that had been defined by Ernst Wertheim 1912. Meigs had performed abdominal surgery with hysterectomy and extended the parametrial dissection to the pelvic sidewall where he stressed the importance of systematic lymph node dissection in the pelvis. A five year survival rate of 89,5% for patients with stage I disease and 63% for patients with stage II disease was presented (43). Ever since modifications have been made both on the extension of the hysterectomy towards less radical surgery and the operation modalities have shifted from open to minimally invasive surgical (MIS) technique. With laparoscopic surgery, introduced in the nineties, there was a significantly longer operation time but the patients lost less blood and had shorter hospital stay (44). Despite some advantages for the patients, the method did not gain popularity worldwide, probably because of limitation such as a prolonged learning curve for the operator, long operation time and ergonomic issues.

Robotic assisted laparoscopic surgery (RALS) was accepted 2005 in the US and rapidly became widely accepted and introduced into gynecological oncology. Observational studies confirmed advantages in form of shorter hospital stay, less bleeding and acceptable operation time as compared to conventional laparoscopy (45-47). The data on oncological safety consisted until last year of retrospective observational cohorts of 100-500 patients with a follow up time from 30-60 months. These studies are consistent in regards to recurrence rate of 9-13% for patients operated on with robotically assisted radical hysterectomy (RRH) with no statistically significant difference when compared to abdominal radical hysterectomy (ARH) cohorts (48-51). The results from the LACC trial, a phase III, multi-center non-inferiority Randomized controlled trial to comparing the outcomes of Laparoscopic Radical Hysterectomy (either conventional or robotically assisted) to ARH was published last year. The results showed a HR of 3.74 (95% CI 1.63 – 8.58, p = 0.002) for recurrences in the laparoscopy group and HR 6.00 (95% CI 1.77 – 20.3, p = 0.004) for OS in favor of ARH after a follow up time of 2,5 years. There was no statistically significant difference between the groups on quality of life or complications (52). These shocking results were further strengthened by a register study including almost 2500 patients in the US showing an increased mortality in women with stages IA2 and IB1 who were operated with minimally invasive surgery, HR 1.65 (95% CI 1.22

- 2.22; p = 0.002) for death after the introduction of RALS (53). These results have questioned the modern practice that in most western countries consists of RALS as a routine for the treatment of patients with cervical cancer. The criticism on the LACC study has been that it mainly consisted of laparoscopic surgery (86%) and not RALS (14%) and the low recurrence rates in both these studies do not resemble those from previously mentioned retrospective studies. Irrespective of that, as the first large prospective randomized trial the results of LACC have to be taken seriously and further studies on RALS and ARH are expected.

Fertility sparing surgery has been in the treatment arsenal for patients with early stage cervical cancer for decades. It has either been performed as a cone biopsy or as a radical trachelectomy where the uterine cervix is removed together with surrounding pericervical tissue preserving about 0,5-1 cm of the cranial cervix including isthmus uteri that is attached back to the vaginal mucosa. As this procedure is relatively uncommon, no randomized controlled trials or large prospective trials have been published. The data available are from retrospective series, the largest from 2016 that reviewed the literature for patients with stage IA-IIA1 disease. A recurrence rate of 5% for abdominal radical trachelectomies from a total of 660 patients versus 6% in the 238 patients that had been operated by MIS was found (54).
In a conclusion ARH with pelvic lymph node dissection is to be considered the current gold standard for treatment of patients with early stage cervical cancer. Oophorectomy is not recommended unless for patients with larger tumours > 4 cm or with other histology than squamous cell carcinoma as metastasis is uncommon with squamous cell and smaller tumours of adenocarcinoma (55).

2.6.2 Sentinel node biopsy

Sentinel node biopsy (SNB) is a well-established diagnostic method in oncological surgery that identifies the first lymph node in the lymphatic chain from the organ of interest. In gynecological tumours it has been substantially studied and since 10 years adapted for vulvar cancer and recently even endometrial cancer (56-59). The main goal of SNB is to retain safe lymph node diagnostics but diminish morbidity by omitting complete lymph node dissection in SNB negative patients (60-63). The prerequisite for the acceptance of such method is a high diagnostic accuracy and low detection failure, where the most important aspect of the diagnostic performance would be high sensitivity and high NPV. A new technique based upon fluorescent dye, Indocyanine green (ICG), and near infrared imaging has been studied on

patients with endometrial and cervical cancer and appears to be both feasible and superior to older tracers for sentinel node detection, yielding up to 100% unilateral and >90% bilateral SNB (62, 64, 65). Further advance with ICG is the possibility to detect and follow the lymphatic vessels to the SNB nodes thereby creating a more comprehensive mapping, (Figure 5) (66). In a review from 2015 of the diagnostic studies available on SNB for cervical cancer patients the authors concluded that pooled sensitivity (n=1275) was 94% and NPV of 91-100%. With a combination of following factors: stages IA2-IIA, tumour size < 40 mm, no suspicious metastatic disease pre or peroperatively and bilateral negative SNB after ultra-staging (thinner slides from the lymph nodes for histological evaluation) there was only 1 false negative result (0.08%) (67). Due to lack of large prospective randomized trials, the question whether lymph node dissection is therapeutic or only diagnostic still remains unanswered causing hesitation to abandon the radical lymph node dissection and adapt the seemingly reliable SNB technique.

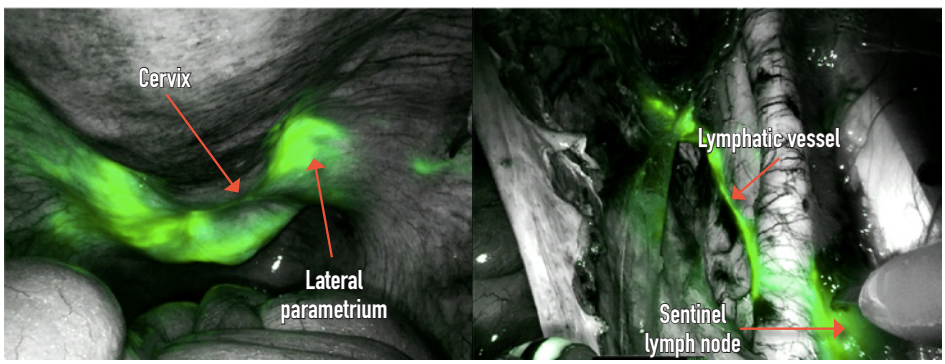


Figure 5. The lymphatic mapping with near infrared imaging and ICG in a patient with early stage cervical cancer. 5a; a trans peritoneal view of the ICG uptake from the backside of the uterine cervix. 5b; following the lymphatic vessels along the iliac vessels on the right pelvic wall, closest in view an ICG positive lymph node.

2.6.3 Radiation and chemotherapy

Radiation therapy is since the 1960´s the cornerstone of treatment for locally advanced cervical cancer. To date radiation-chemotherapy (RCT) combining external beam radiation therapy (EBRT), concomitant Cisplatin and intracavitary radiotherapy (brachytherapy) is standard treatment for patients with advanced stages ≥ IB2, with some exceptions for selective cases with stage IIA1. A total dosage of EBRT 45-50 Gy is the standard, given in daily fractions of 1.8-2.0 Gy as this dose has been shown to be sufficient to eradicate micro-metastases with acceptable toxicity (68). The treatment is directed over the pelvic region covering the genitalia and lymph

nodes, with cranial border around lumbar vertebrae 4-5. Brachytherapy is a key component of the radiation therapy to gain local control over the tumour (69). By a concentration of high dose radiation centrally around the probe adjacent organs can be spared. The treatment is targeted with the help of modern MRI led 3D technique adjusting the field to shrinking tumour volumes during the treatment period (70). In Sweden a total dose of 55-60 Gy divided between (EBRT) and brachytherapy is used for patients with metastasis < 2 cm, but a higher total dose of 60-64 Gy is recommended for larger metastasis > 2 cm. Furthermore, a boost over lymph nodes with suspected metastasis is given (36). Based on the prospective RTOG trial (n=402) and data from other prospective trials, the addition of weekly concomitant Cisplatin treatment to radiation therapy increases the OS for patients with cervical cancer and should be standard of care (71), with recommended 6 cycles of treatment as the number of cycles given < 6 is a predictor for worse survival (72).

2.6.4 Treatment according to disease stage

As previously stated 1/3 of cervical cancer cases in Sweden are found through screening. In a microscopic disease up to stage IA1 surgery with cone biopsy or simple hysterectomy is sufficient, as long as no lymph vascular space invasion (LVSI) is present. In stage IA1 with LVSI present, surgical lymph node diagnostics are recommended and for IA2 surgery with radical hysterectomy or trachelectomy together with lymph node dissection is the treatment of choice, as evidence is lacking for less radical surgery from stage IA2 (73, 74). For stage IB1 fertility sparing surgery can be offered in cases of small (<20 mm) tumours if no metastases are found in the pelvic lymph nodes and high-risk histology such as neuroendocrine tumour is excluded (75, 76). Due to increased risk for long term morbidity following RCT, patients with stage IB1 and selective cases of IIA1 will be recommended radical hysterectomy and pelvic lymphadenectomy, even though primary RCT is reported to be equally effective in this group of patients (77). Combined RCT is standard of care for women with disease stage ≥ IB2 in Sweden.

Patients with unfavorable histological features after surgery are subjects to adjuvant RCT. A metastasis in the lymph nodes and neuroendocrine histological subtype are definitive high risk factors. If more than one of following intermediate risk factors is present: > 1/3 deep stromal invasion, positive LVSI or the tumour size > 4 cm at histology, adjuvant therapy should be considered even if no metastases are found in the lymph nodes (78-80).

All treatment options except fertility sparing surgery for tumours < 2 cm causes the patients secondary infertility.

2.7 PROGNOSTIC FACTORS AND SURVIVAL

The strongest prognostic factor in patients with cervical cancer is the disease stage and presence of metastases in the lymph nodes at the time of diagnosis (10, 81). Tumour size is also an independent prognostic factor with increasing risk for recurrence and lymph node metastases with increasing tumour size (82). A prospective cohort study on 366 surgically treated patients with stage IB1 elegantly showed that the 5-year recurrence free survival rate decreased significantly with increasing tumour size from 94.2% for tumours ≤ 2.0 cm to 82.8% for size 2-4 cm and 67.2% for tumours > 4 cm, ($p < 0.001$) (76). Other factors than lymph node metastases affecting survival in early stages have been suggested; (LVSI), depth of invasion to the cervical stroma, parametrial invasion, certain histological subtypes and age (78, 82-86). Results are inconsistent for age and LVSI and it is possible that LVSI and depth of stromal invasion are more of a surrogate for the risk for lymph node dissemination, than a separate independent risk factors.

According to FIGO 26th annual report from 1999-2001 including almost 15000 women worldwide the total 5 year OS irrespective of disease stage was 69.5% whereas in the most common stage IB1 survival was 89.5%. For all stages, where metastases were found in the lymph nodes, the Hazard Ratio (HR) of death within 5 years from diagnosis was 3.3 (95% CI 2.8-4.0), for stage IB1 the OS dropped from 94.5% to 75.9% with findings of lymph node metastases with HR 4.7 (95% CI 3.5-6.4) for death (10). According to a report from National Board of Health and Welfare (Socialstyrelsen) published 2018, the relative 5 year survival is 76.1 % and 10 year survival 69.7% for women diagnosed with cervical cancer in Sweden (87).

2.8 DIAGNOSTIC IMAGING

2.8.1 Magnetic resonance imaging and Computed tomography

An overview of numerous studies on MRI for the preoperative evaluation of patients with cervical cancer is presented in Table 2. Majority are retrospective single center series focusing on the performance of MRI and in some cases even CT with overall accuracy (correctly classified cases) of staging using histology as gold standard. A good overall accuracy between 77- 90% for MRI with worse performance of CT 65-75% is reported where MRI has both a high sensitivity and specificity to find parametrial disease (88-90). Different statistical methods have been applied for tumour visualization in different studies but overall the detection rate lies between 65-91% (Table 2). At least two of the larger studies reported poorer performance of MRI to detect smaller tumours < 10 mm (91, 92).

The ACRIN/GOG study was the first large prospective multicenter study in the US to evaluate the accuracy of MRI and CT as compared to clinical staging in patients with cervical carcinoma. With intention to include > 450 patients with FIGO stadium ≥ IB planed for surgery the study was closed ahead of schedule with only 208 patients enrolled because of the increasing reliance on CT and MRI during the clinical workup of patients introducing bias to the preoperative staging and secondly due to the slow rate of recruitment. The results showed poor sensitivity 26% of FIGO clinical criteria for correctly diagnosed stadium ≥ IIB in operated patients but a specificity of 99%, in comparison to CT with a sensitivity of 42% and specificity 82% while MRI had a sensitivity of 53% and a specificity of 75% with no statistically significant differences between the methods (92, 93). As the results indicated poorer performance for MRI compared to the previous retrospective trials to detect parametrial invasion the authors suggested that it could be due to smaller tumours included in the ACRIN/GOG study compared to the previous studies, furthermore, that MRI performed worse in these cases. This conclusion is supported by Bipat et al who in a review of > 50 articles 2003 had questioned the additional role of MRI in the staging of early stage disease (94). Similar results came from a retrospective study on 101 patients where MRI was reported as equal to CT and not superior to clinical examination for staging (95). In a meta-analysis from 2103 comparing MRI and clinical staging for advanced stage disease ≥ IIB, the results from 40 studies (n=3254 patients) were pooled, showing a sensitivity of 84% vs. 40% and specificity 92% vs. 93% for parametrial invasion, sensitivity 79% vs. 53% and specificity 93% vs. 97% to correctly stage advanced disease (bladder, rectum) for MRI and clinical examination respectively (96). For preoperative lymph node assessment MRI has a reported sensitivity 24-100% and specificity 84-99%, (Table 2). The performances of CT and MRI for detection of metastases in the lymph nodes were similar in these studies (89, 92, 97, 98).

Diffusion weighted imaging has appeared as a complement to conventional MRI. It is based on free diffusion of water molecules in tissues and according to some evidence increases the accuracy for local assessment (99). Partly by using so called Apparent diffusion coefficient that is lower in tumours compared to healthy cervical stroma and seems to have a predictive value for finding disease in the parametrium as well as for the risk of recurrence (100, 101). Furthermore, the addition of Diffusion-weighted sequences seems to increase the security of evaluation for the less experienced MRI reader (102).

To summarize the above, MRI has been adopted in most western hospitals as part

Table 2. Overview over some of the main studies assessing accuracy of Magnetic Resonance imaging for the evaluation of patients with cervical cancer. Data adapted from(1, 88-92, 95, 98, 104-106)

				Tumor detection	Stromal invasion	Staging		Parametrium		Lymph nodes		
First author	Year	n	Study type	Accuracy	Accuracy	Accuracy	Sens	Spec	Accuracy	Sens	Spec	Accuracy
Hricak	1988	57	R	91%	79%	81%	89%	87%	88%	100%	96%	96%
Kim	1993	99	R	75%	76%	77%	20%	97%	87%	24%	99%	88%
Subak	1995	71	R	N/A	76%	90%	100%	94%	94%	62%	91%	86%
Hricak	2005	166	P	AUC 0.88	sens 83%, spec 22%	42%	53%	75%	70%	37%	94%	75%
Sahdev	2007	150	R	sens 65%, spec 77%	N/A	kappa 0.54	100%	98%	98%	37%	92%	N/S
Hancke	2008	101	R	N/A	N/A	58%	61%	63%	61%	36%	84%	N/S
Fischerova*	2008	95	P	83%	N/A	N/A	50%	98%	95%	N/A	N/A	N/A
Testa*	2009	68	P	sens 88%, spec 51%	sens 94%, spec 85%	N/A	40%	89%	85%	27%	96%	82%
Epstein*	2013	182	P	sens 67%, spec 89%	sens 89%, spec 88%	N/A	69%	92%	90%	11%	N/A	N/A
Zhang	2014	125	N/S	kappa 0.88	N/A	N/A	N/A	100%	N/A	28%	86%	78%
Moloney*	2016	33	P	N/A	sens 80%, spec 50%	N/A	40%	86%	79%	N/A	N/A	N/A

Accuracy = correctly classified cases/ total number of cases sens= sensitivity, spec = specificity, R= retrospective, P= prospective, N/A = not assessed, N/S = not specificed
*Studies comparing MRI and US, results for US presented in Table 3

of the routine clinical workup of patients with cervical carcinoma with evidence supporting improved diagnostic accuracy especially for advanced disease stages > IB1 when compared to FIGO´s clinical criteria alone. There is some inconsistency regarding accuracy of tumour detection from different studies indicating worse performance for smaller tumours < 10 mm as well as low sensitivity for the evaluation of lymph node metastases (96, 103). Plain CT scan in the diagnostic work-up of patients with cervical carcinoma is unmotivated as no obvious advantages are gained with CT as compared to MRI for this patient group (94).

2.8.2 Positron emission tomography (PET)

PET performed with 2-fluoro-2-deoxy-D-glucose labeled with the positron emitter fluorine-18 is a molecular functional imaging modality (FDG PET/CT). Through increased glucose metabolism of cancer cells it enables detection of metastases > 5mm in size that otherwise would not be considered pathological on plain CT or MRI (107). However, FDG PET/CT has limited value in the primary staging of low volume local disease in patients with early stage cervical cancer, even when micro-metastases are present (108). According to a meta-analysis from 2017 the accuracy for detecting lymph nodes metastasis with PET/CT (n=22 studies) is higher in advanced stage disease as compared to early stage disease (AUC 0.95 versus AUC 0.76, p = 0.03) (109). In Sweden PET/CT is recommended as a complementary examination for patients where advanced disease is suspected.

2.8.3 Ultrasound

In 1950 John Julian Wild with coworkers published an article on unidirectional A-mode ultrasound waves to explore the thickness of surgical intestinal material (110, 111). During their research Wild and Donald Neal learned that malignant tissue in the bowel had a hyper echoic character seen with ultrasound waves and did not contract and relax in the same manner as healthy bowel (110, 112). Some years later or 1958 when diagnostic scanners had been developed Professor Ian Donald presented his research in the Lancet about investigation of abdominal masses by Pulsed Ultrasound, thereby introducing the method in Obstetrics and gynecology (110, 113). Ultrasound is now the most widely available and thoroughly studied imaging modality used by obstetrician and gynecologists around the globe. The technique is introduced to all trainees from the first day working in the field. Compared to MRI ultrasound has both some advantages and disadvantages. In regard to the diseases in the pelvis a closer look and more dynamic image is achieved, being able to adjust the image quality in real time and zoom in on areas of inter-

est. For the patient it is safe with few or any contraindications other than possible discomfort during the examination. The negative aspects are the difficulties in standardizing image acquisition, as this is operator dependent. Moreover, transvaginal ultrasound must be complemented by transabdominal scanning to get an overview of the pelvis in the manner that is possible by MRI or CT.

2.8.4 Ultrasound on patients with cervical cancer

In a case report from may 1992 the authors describe the aid in using transvaginal ultrasonography (TVS) in the staging of a woman with cervical cancer as the ultrasound revealed parametrial involvement and lymph node metastases in the pelvis, not found by clinical palpation (114). That same year Innocenti and coworkers published a prospective study on a cohort of 124 women with surgically treated cervical cancer. For the extent of parametrial involvement, the sensitivity of ultrasound evaluation was 78% and the specificity 89% compared to clinical evaluation with sensitivity 52% and specificity 92% (115). Since then the ultrasonography technique has improved dramatically with high quality grayscale imaging available as well as additional modalities such as Doppler, 3D US and CEUS.

Testa and co-workers found that vascularity was seen in nearly all lesions of cervical cancer (116) with both two dimensional (2D) color Doppler and 3D, confirming Alcazar's previous results on high blood flow mainly in squamous cell types and poorly differentiated lesions (117). In 2010 Epstein and colleagues related different histology types of cervical cancer to findings with TVS where adenocarcinomas were more difficult to see with grayscale ultrasound compared to squamous cell cancer because the lesions were iso-echoic to the surrounding normal tissue (Figure 6). Further results were that virtually all tumours in the uterine cervix were richly vascularized which facilitated detection and assessment (118). To further study the agreement between TVS prior to surgery and histology after surgery Gaurilcikas with co-workers published a prospective series on 18 patients with FIGO stage IB1-IIA cervical cancer. They found good agreement for tumour size and parametrial infiltration (100%) but a worse correlation for the assessment of tumour invasion of the cervical stroma (119).

In order to standardize the evaluation of patients with cervical cancer, Fischerova wrote a review article describing a method for ultrasonography examination of these patients (120). The importance of adequate equipment is stressed as well as the knowledge on the expected dissemination pattern. By performing a structured examination all the important aspects of tumour dissemination are covered. The

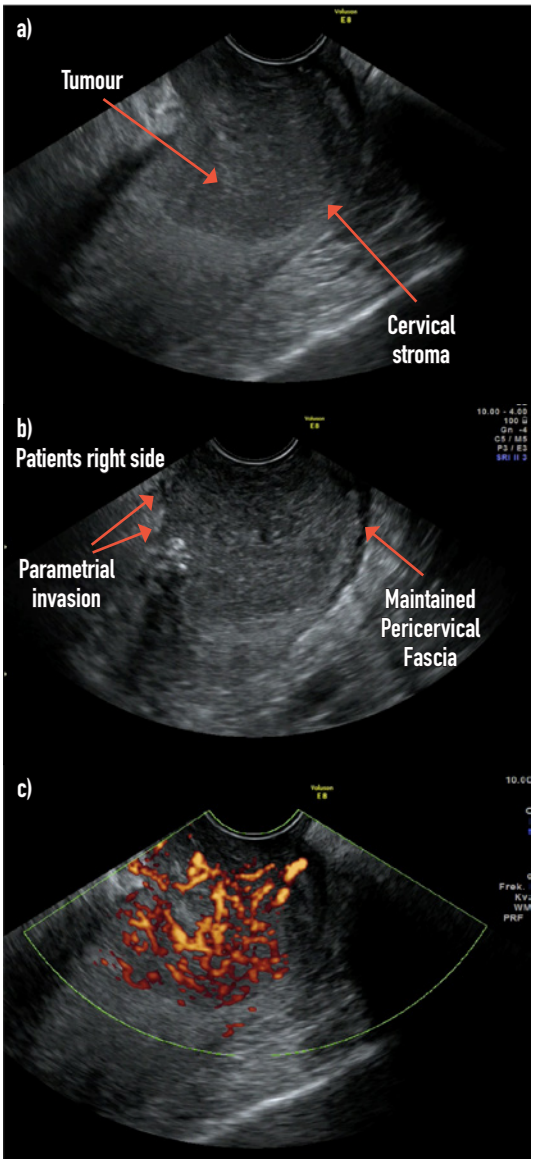


Figure 6. A 63 years old woman with cervical cancer, stage IIB. 6a: US grayscale image in sagittal plane showing hypoechoic tumour filling out almost the whole cervix with minimal cervical stroma seen on the back side 6b:Transverse section of the same tumour showing parametrial invasion on the patients right side but maintained paracervical fascia on the patients left side (arrow) (arrow). 6c: 2D power Doppler in sagital plane, abundant vascularization the indicates invasion anteriorly towards the bladder.

recommended probe is a micro convex array probe either transvaginally or transrectally to evaluate if tumour is seen or not; followed by description of the growth pattern; exophytic or infiltrative. Measurements are done in three dimensions (cervico-fundal, anterio-posterior and lateral), in cases when fertility sparing surgery is considered the distance of the cranial tumour boarder to the internal cervical os is measured. The depth of stromal invasion (≤ 1/3 or 2/3 or > 1/3 or 2/3) is assessed as well as the involvement of the parametrium when the pericervical fascia is disrupted. Parametrial invasion can be graded according to Figure 7. Power Doppler examination should complement the grayscale image to estimate the amount of vascularization with color score. Finally, assessment of overgrowth onto adjacent pelvic organs, hydroureters, hydronephrosis and any bulky pelvic, paraortic, inguinal lymph nodes is done by combining systematic transrectal/transvaginal ultrasound examination with trans abdominal scanning using a linear probe (120). For the US examination of the patients included in the studies of this thesis, Fischerovas method was followed.

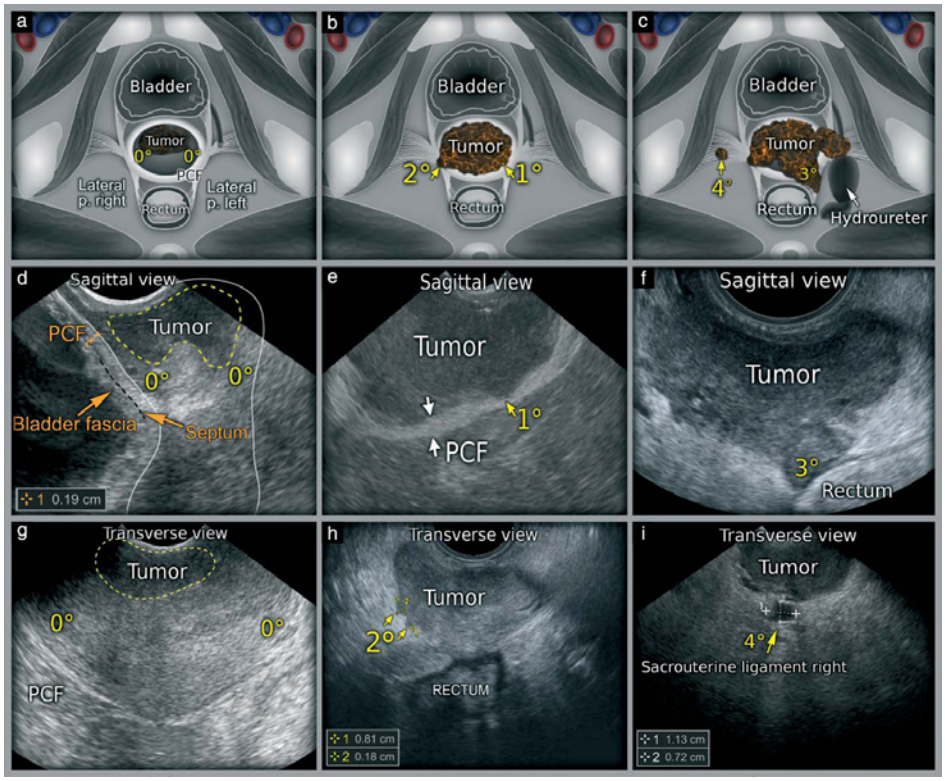


Figure 7. Grading of parametrial infiltration by transvaginal/transrectal sonography. (a–c) Schematic diagrams showing: (a) intact pericervical fascia (PCF) (Grade 0), lateral parametria (lateral p.) and PCF; (b) parametrial infiltration Grade 1 (disrupted PCF but without progression through the fascia into parametria) and Grade 2 (incipient infiltration of PCF); note that the incipient infiltration is characterized by discrete breaks in the PCF with very fine hypoechogenic prominences into the parametria; (c) nodular infiltration (Grade 3) and discontinuous parametrial infiltration (Grade 4). (d–i) Ultrasound images showing: (d) in the sagittal view, hyperechogenic PCF (Grade 0) located between the cervix and hyperechogenic bladder visceral fascia (black dashed line corresponds to the vesicocervical space also known as the septum); (g) in the transverse view, intact PCF surrounding the cervical stroma dorsally; (e,f,h,i) parametrial infiltration Grades 1–4. Reprinted with permission from Ultrasound in Obstetrics and Gynecology Vol. 38, No. 3: 246-266.

2.8.4.1 Ultrasound and MRI

There are several studies published comparing the accuracy of MRI and US in patients with early stage disease, the most relevant results are summarized in Table 3. The three largest prospective studies have all presented similar results on tumour detection rate. In a study on 68 patients, Testa and colleagues found 93% detection rate of tumour with US compared to 88% on the MRI, further they found similar accuracy of the methods for assessment of deep stromal and parametrial invasion (Table 2 and 3) (105). In Fischerova and colleagues paper on 95 surgically treated patients they found that TRS was better than MRI at detecting small tumors with

detection rates 90% versus 81% for tumors <10 mm (p=0.05), whereas for parametrial invasion equal specificity (100 vs. 98%) but higher sensitivity (83% vs. 50%) was detected for US (104). Further data from a larger multicenter study by Epstein and coworkers on 182 women with early stage disease strengthens the evidence for the high accuracy of US for tumour visualization, reporting that ultrasound is equal or better than MRI in detecting residual tumors even after cone biopsy, for deep stromal and parametrial invasion, however, as in Testa´s study both modalities were limited for the diagnostics of lymph node metastases (1, 105).

There are few studies published on the performance of conventional US on patients with advanced disease stages. The PRICE study included 88 women with diseases stages IB2-IVA who received neoadjuvant RCT therapy before surgical treatment. US examination with 2D, 3D and CEUS were performed before and after neoadjuvant treatment to evaluate if any US modality was useful to monitor treatment response and detect residual disease after NACT. None of the different modalities turned out accurate enough to detect microscopic residual disease, of all modalities 2D US with power Doppler had the best performance with sensitivity 63% and specificity 70% (121).

2.8.4.2 Three dimensional ultrasonography (3D)

When the three-dimensional ultrasound vaginal probes emerged there were hopes on its usefulness on gynecological patients. The examination is done in the same manner as TVS where images are collected through one plane with 2D US. Before collecting the volume the image is optimized with the organ of interest is centered centrally located. The volume is then acquired through automated sweep holding the probe completely still. The quality of the volume is dependent on the quality of the 2D image, motion artifacts, the size of the volume and the number of voxels which are factors that can be adjusted before the sweeping is done (122). The resolution in the collected plane is always good, but the quality of the reconstructed plane is highly dependent on the quality of the volume collected. From the volume planes that can normally not be seen with 2D, reconstructed planes are built such as the important coronary view. By using 3D power Doppler the vascular tree can be visualized in three dimensions with a possibility of removing the grayscale echoes (123). The vascularization within a volume can be assessed with three-dimensional power-Doppler (3D-PD) by calculating the vascularization index (VI), vascularization-flow index (VFI) and flow index (FI) with the help of software package. There is some uncertainty on what exactly is measured by 3D-PD but VI is supposed to

Table 3. Overview of the main studies on the accuracy of ultrasonography for the assessment of patients with early stage cervical cancer. Data adapted from (1, 104-106, 115)

				Tumor detection	Stromal invasion	Staging		Parametrium		Lymph nodes		
First author	Year	n	Study type	Accuracy	Accuracy	Accuracy	Sens	Spec	Accuracy	Sens	Spec	Accuracy
Innocenti	1992	124	P	N/A	N/A	83%	78%	89%	87%	N/A	N/A	N/A
Fischerova	2008	95	P	94%	N/A	N/A	83%	100%	99%	N/A	N/A	N/A
Testa	2009	68	P	sens 93%, spec 50%	sens 100%, spec 75%	N/A	60%	89%	87%	9%	100%	82%
Epstein	2013	182	P	sens 90%, spec 97%	sens 88%, spec 93%	N/A	77%	98%	97%	8%	N/A	N/A
Moloney	2016	33	P	N/A	sens 80%, spec 50%	N/A	20%	89%	79%	N/A	N/A	N/A

Accuracy: correctly classified cases / total number of cases, sens = sensitivity, spec = specificity, p= prospective, N/A = Not assessed

quantify the number of color voxels in a volume indicating the density of vessels in the tissue of interest. FI represents the intensity of flow in the region of interest (ROI) at the time of the three-dimensional examination. VFI is a combination of vascularization and flow information (124, 125).

As already discussed in previous chapter cervical tumours are richly vascularized in contrast to the fibrotic cervical stroma. There is substantial research done on the vascularity aspect of 3D US where it has been confirmed that all three vascularity indices are higher in patients with invasive cervical carcinoma as compared to healthy cervical tissue (116, 126-128). Two studies have focused on the relationship between vascularization, vascularity indices and prognostic histo-pathological factors where the results suggested higher 3D-PD vascularity indices in more advanced tumours, furthermore, higher vascular indices in poorly differentiated tumours (126, 127). Despite that, no correlation was found between 3D-PD indices and lymph node metastases in neither of these studies. Even if these studies were relatively small, the results were consistent regarding vascularity indices and lymph node metastases. No study has yet evaluated 3D parameters for the prediction of deep stromal invasion or parametrial invasion. Research on 3D US and cervical cancer has focused as well on the possible predictive value for patients that are subject to NACT before surgery or primary RCT with negative results. FI was suggested as a predictive marker for NACT responders, (129) however, neither in the prospective PRICE study nor another smaller study were 3D vascular indices found useful to monitor response to NACT or RCT in patients with advanced disease (121, 130).

Limited but promising results are available on the accuracy of 3D US for staging. In a small sample of 24 patients with locally advanced disease, 3D US had comparable overall accuracy to MRI 62.5% versus 66.7% respectively with histology as gold standard (131). Chiappa and colleagues examined 29 patients using MRI as a reference standard and found a comparable, moderate correlation between 2D US, 3D US and MRI with the best agreement (81%) in evaluating right lateral parametrium in the middle part of the cervix (132). Still another study on 40 patients reported 80% overall agreement for parametrial invasion between 3D US and MRI with a κ =0.60 and very good κ = 0.84 with 97.5% agreement for bladder involvement (133).

2.8.4.3 Contrast enhanced ultrasonography (CEUS)

CEUS makes it possible to see small blood vessels not visualized with conventional 2D PD US (134). Tumours are characterized by neovascularization and it has been

documented that cervical tumours are often well vascularized making the CEUS particularly interesting for tumour evaluation. There are a couple of different contrast agents available but SonoVue ® is by far the most commonly used one with a well documented safety profile (135, 136). It is composed of a gas micro bubbles containing Sulphur hexafluoride, stabilized by a monolayer of phospholipids. It is administrated intravenously as a bolus followed by saline injection and during a window of 2-3 minutes ultrasound videos are collected with a split screen for the contrast view and the usual B-mode view (137). The interpretation and recording of CEUS images demands special software installed on the ultrasound machine. The contrast agent is cleared from the body through the lungs with breathing and does not affect the kidneys or liver. CEUS has improved the diagnosis of liver tumours, mostly in the differential diagnosis between metastatic lesions and benign lesions (138-140). According to the European federation of societies for ultrasound in medicine and biology (EFSUMB) there are no established indications for using CEUS in gynecological diagnostics to date and the clinical value of the method in the assessment of cervical tumours is uncertain (141). Testa and colleagues did a small descriptive study including 10 patients with cervical carcinoma examined with CEUS, where the method improved the demarcation of the tumour borders in 40% of cases (142). When the work started on this thesis, no studies were available on the contrast kinetics in patients with cervical cancer and as CEUS has a potential as a surrogate for vascularization in tumour tissue, it was important to further explore the method.

Since then two publication have emerged both from Zheng and colleagues showing that contrast kinetics are different in tumorous tissue compared to myometrium in the uterus, moreover, that micro vascular density found on histological examination after surgery correlates to higher contrast intensity as measured by CEUS. In a recently published paper CEUS (abdominal probe) was compared to MRI in the evaluation of local invasion of cervical cancer in 108 patients with advanced stages IIA2-IVB. The results showed a strong correlation in size measurements r=0.84-0.88 between the two methods and a moderate (parametrial, vagina) to good (uterine corpus, bladder and rectum) concordance for the evaluation of local spreading to adjacent organs (143).

2.8.4.4 Strain Elastography

Ultrasound strain elastography (SE) is a technique that was proposed in the nineties by Ophir and colleagues as a complement to conventional ultrasonography (144). Taking advantage of the different compliance of human tissue the technique col-

lects information on the elasticity of the tissue examined. In a simplified way it can extract the findings of clinical palpation into an image thereby more objectively quantify the clinical findings. The image of 2D SE is presented with color range superimposed on a grayscale ultrasound image, (Figure 8) where differences in tissue strain appear with different colors. Red is the softest tissue that changes form with pressure, blue is the hardest with the least strain and green is in between (145). With extra software package, further semi-quantitative analysis on the SE images is possible such as calculating strain ratio or size ratio as compared to B-mode images.

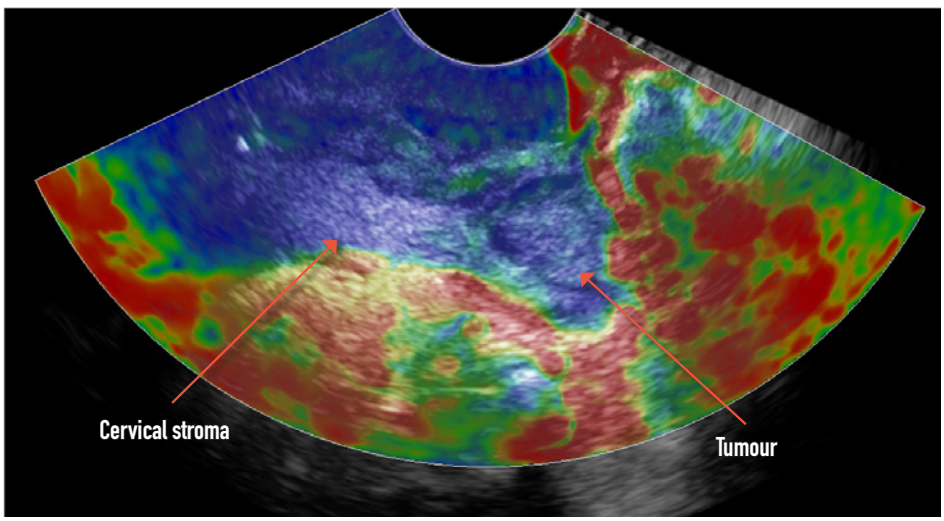


Figure 8. Strain elastography image from an examination of a patient with stage IB1 cervical cancer. Cervical stromal tissue and tumour are seen with different shades of blue with the softer surrounding tissue in red and green colors. The grayscale image is used in the background to build on the elastography images.

SE has been extensively examined in patients with breast cancer where it can complement the conventional B-mode US to differentiate between benign and malignant lesions (146-148). Furthermore SE in addition to conventional US seems to increase accuracy of targeted biopsies in patients with suspected prostate cancer (149, 150). Thomas and colleagues studied uterine cervixes in healthy women and women with cervical cancer (n=113) and found that normal healthy cervical tissue is of medium hardness and stays unchanged with age. They concluded that the SE helped to outline malignant tumours from healthy tissue as there was a significant difference in stiffness of the tumour lesions (151). This finding was confirmed with the results of Lu and colleagues who in a prospective study on a mixed group of 84 women with benign and malignant tumours in the uterine cervix showed a

positive predictive value (PPV) of 85,7% and negative predictive value (NPV) 81,0% for higher elasticity score 4-5 to distinguish malignancy from benign disease. They further tested strain ratio with promising result of around 90% PPV and NPV for a cut-off 4.525 to distinguish between malign and benign lesions in uterine cervices, thereby confirming the same cut-off ratio that had been suggested by Sun and colleagues from a previous prospective series of over 100 patients with mixed cervical lesions (152). A feasibility study on the SE examination of 36 patients with locally advanced cervical cancer before and after combined RCT showed that the patients with complete response after given treatment had greater decrease in strain ratio during and after treatment compared to those who were partial responders (153). No studies have compared the accuracy of tumour assessment locally in the pelvis with SE, neither subjective evaluation or size measurements compared with US alone.

2.8.5 Reproducibility

For all different diagnostic imaging, inter-observer reproducibility is a concern. Most studies report the accuracy of one or two different readers where reproducibility is not always evaluated. At least two prospective studies have confirmed a good inter-observer and intra-observer agreement for 3D US in cervical cancer patients for measurements of vascularity indices (154, 155). In the ACRIN/GOG study, inter-rater reliability was moderate for staging (κ=0.44), fair for tumour visualization (κ=0.32) and poor for parametrial invasion (κ=0.11) with retrospective readings by four experts on MRI (156). No published studies on inter-rater reliability (reproducibility) for conventional 2D US or studies comparing the reproducibility of MRI and US for diagnostic evaluation of women with cervical cancer have been identified.

2.9 SUMMARY

The staging of newly diagnosed patients with cervical cancer remains a challenge as a result of the inadequate clinically based staging system and because available diagnostic imaging methods are insufficient to complete the clinical system. There is substantial evidence for high accuracy of US assessment with regard to tumour visualization, evaluation of deep stromal invasion and there seems to be a high sensitivity for finding parametrial infiltration in women with early stage cervical cancer. For obvious reasons, there is scares evidence for the accuracy of all imaging modalities with regard to assessing advanced disease, as these women will not undergo surgery. Due to a good overview of the pelvis, high tissue resolution and evidence

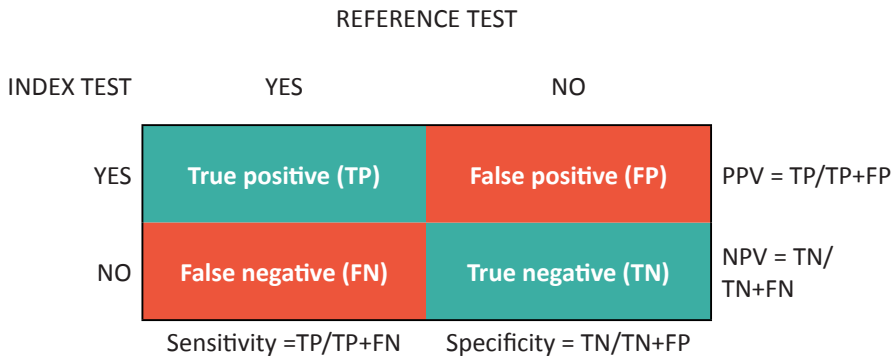
for its accuracy, MRI has become the complementary method of choice for cervical cancer patients both for treatment triage as well as being an important instrument guiding the 3D brachytherapy (157). The availability of MRI however, remains limited in low resource countries where the incidence of cervical cancer is high (5). The method has its limitations just as the US, such as a tendency to overestimate the size of smallest tumours in the cervix as well as missing 20% or more of lymph node metastases even with the added Diffusion weighted sequences (1, 92, 158). Another diagnostic dilemma is when surgical margins from a diagnostic cone biopsy are not disease free, the patient will be assigned stage IB1 even if no macroscopic lesion can be identified. In these cases, assessment of residual disease by imaging or clinical examination is difficult. The patients are according to the actual recommendations often going through more extensive surgery than necessary. This is an important group, as in Sweden about 35% of the patients are still in reproductive age where some strongly wish to preserve fertility. For this reason it is important to further study the value of CEUS or SE in addition to conventional US on the smallest tumours in terms of characteristics and accuracy. It would also be an advantage to be able to predict which patients are at increased risk for lymph node metastases by identifying some preoperative factors on US. To explore the full potential of ultrasound for patients with cervical cancer it is important to examine if new modalities are applicable in our settings, what are the most beneficial aspects of the methods, furthermore, to examine if US and MRI are comparable in terms of reproducibility for local tumour assessment.

2.10 METHODOLOGY IN DIAGNOSTIC STUDIES

2.10.1 Sensitivity/specificity

Diagnostic studies aim to evaluate the potential of a test to separate a diseased from non-diseased objects. The term accuracy refers to the ability of the test to correctly find the objects that truly have a condition (true positive) and those who truly don´t have the condition (true negative). The test under evaluation is referred to as **index test** and to be able to assess it´s accuracy an accepted standardized test is used as the **reference test**, often the gold standard within the field of interest. There are different ways to present the accuracy of test. The ability of a test to find a disease or a condition is called sensitivity whereas the ability of the test to separate the objects without disease from the rest is called specificity. A predictive value can be calculated both negative and positive that describes the index test ability to predict the value of positive or negative results in a certain population. The prev-

alence of the disease in the population assessed affects the positive and negative predictive value and to a less extends the sensitivity and specificity of the index test under evaluation. A test that works well in a selective population of patients may not at all be applicable as a screening test where the prevalence is low. The perfect test would have 100% sensitivity and specificity, which is rarely the case. Depending on the indication for the test a compromise has to be done between the two. A common way to present the results in a diagnostic accuracy study is by a 2x2 table where the reader can calculate sensitivity, specificity, PPV and NPV;



Another way to refer to accuracy is by correctly classified cases where the total number of true negative and true positive cases is divided by the total number of cases

$$\text{Accuracy (correctly classified cases)} = \frac{TP+TN}{TP+FP+TN+FN}$$

2.10.2 Receiver operator characteristics (ROC curve)

To compare the diagnostic accuracy of two or more different tests the sensitivity and specificity can be plotted on a graph with the sensitivity on the y-axis and 1-specificity (false positive rates) on the x-axis, called a Receiver operator characteristics (ROC) curve. A diagonal line (reference line) runs in the middle of the graph, tests that lie to the left of the line are better than chance. The best test would have a curve up in the left corner. For each test it is possible to calculate the Area under the curve that represents the overall accuracy of that test. The closer the AUC gets to 1 the higher accuracy for the test. For every single cut-off point on the curve there is a sensitivity and specificity (Figure 9).

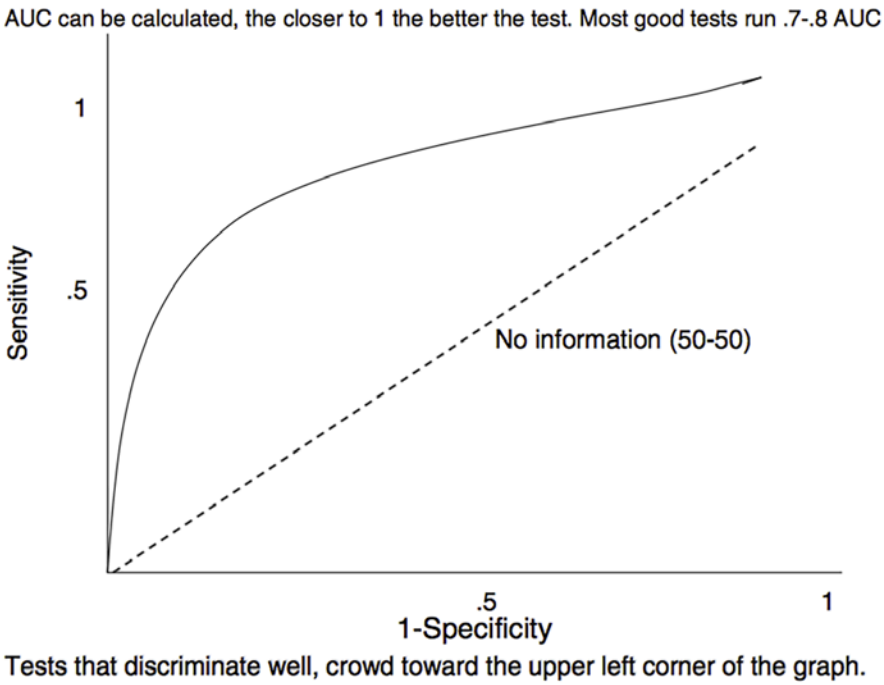


Figure 9. ROC curve showing an example of a test with average AUC the curved line. The dotted diagonal reference line refers to a useless test. Reprinted with permission from (159)

The choice of cut-off point is a trade-off between sensitivity and specificity dependent on what is the most important aspect of the index test. The point that is furthest to the left from the reference line can be referred to statistically as optimal cut-off where you get as high possible sensitivity and specificity, this may however not reflect the optimal cut-off point from a clinical perspective.

2.10.3 Statistical tests

To answer a research question a sample from a population is studied. The null hypothesis (H0) is usually that there is no difference between the study sample and the whole population. The alternative hypothesis (HA) would be that there is a difference. To be able to test your hypothesis a level for significance needs to be set that usually is chosen at 5%. This level marks the risk of making a type I error in the study and to reject the H0 even if it is true. There are number of different statistical tests to use and the outcome being studied is of greatest importance when choosing appropriate test as well as the distribution of the data being tested. In a normally distributed data most usual for continuous outcome the test of choice is students t-test while Mann-Whitney would be applied in a non-normally distributed

continuous data. Chi-square test is used to test hypothesis for ordinal or nominal outcome except when testing unusual outcome or when samples are small fisher´s exact should be applied instead. A good test for binomial data is McNemar. Different statistical methods can also be used to compare means or medians between groups where the choice of test follows the same rules as stated above.

2.10.4 Kappa statistics

When reproducibility of a test or inter-rater reliability is evaluated, it is possible to calculate the overall agreement between two raters as a proportion of the number of cases evaluated. (Po= Cases agreed between 2 raters / total number of cases) However, that does not take into account any skewness, i.e. if the raters agree more on the positive cases or the negative cases. Neither does it consider that agreement between raters can occur by chance (160).

Kappa statistics is a more robust way to calculate agreement as it takes into consideration the part of the agreement between raters that is likely to have occurred by chance. The proportion of agreement due to chance = (proportion of positive tests by both observers) 2 + (the proportion of the agreed negative tests by both observers)2.

The equation for **Cohen Kappa (κ)** that applies when comparing 2 raters is:

$$\kappa = \frac{\text{proportion of agreed cases} - \text{probability of agreement due to chance}}{1 - \text{probability of agreement due to chance}}$$

Fleiss Kappa is comparable to Cohen Kappa but takes into account multiple raters and not only a pair of two (161).

The results of κ are between -1 and 1 but are interpreted for values > 0 according to Brennan and Silman as follows (162):

Poor agreement	< 0.20
Fair	0.21-0.40
Moderate	0.41-0.60
Good	0.61-0.80
Very good	0.81-1.00

An assumption for the application of Kappa statistics is that the raters use the same method to assess the same or different cases. It should not be used to compare the agreement between raters using different modalities as the methods may have different sensitivity and specificity that introduces bias to the measurements.

2.10.5 Bland-Altman plot

It was suggested by Altman and Bland in an article in The Lancet 1986 that correlation was not the best way to assess if two different methods had good agreement as there is often correlation found even with substantial differences in measurements by the methods evaluated. Instead, they suggested a simple way to graphically illustrate what the mean difference (systematic error) between methods is and how the difference for each individual measurement is distributed (random error) between ± 2 SD (95% limits of agreement) from the mean difference. A good agreement has a mean difference as close to 0 as possible representing no bias. If the difference between the measurements of the two methods falls within 95% limits of agreement the precision of the test under evaluation is good (163).

3 AIMS OF THE THESIS

The main overall objective was to improve the diagnostic assessment of cervical tumours in order to optimize and tailor the treatment of each patient. By exploring new ultrasound techniques for the assessment of cervical carcinoma in addition to conventional ultrasound, the specific aims were to study:

1) How well objective 2D and three dimensional ultrasound (3D US) parameters predict deep stromal or parametrial invasion and lymph node metastases, compared to subjective assessment in women with cervical cancer undergoing surgery.

2) The filling pattern, reproducibility and semi-quantitative parameters of contrast-enhanced ultrasound in healthy controls as compared to patients with cervical carcinoma, in addition assess if differences were seen in tumours of various stages.

3) If ultrasound strain elastography could help to more precisely detect and demarcate cervical tumours and evaluate if differences were found in strain elastography features in patients with early and advanced stages.

4) If ultrasound had the same inter-observer reproducibility as MRI in the evaluation of patients with cervical carcinoma.

4 PATIENTS AND METHODS

4.1 STUDY POPULATION

There are two study populations behind this thesis. One international multi-center cohort for study I, including patients from our institution and another cohort only from our institution for study II-IV.

4.1.1 Study population I

The study population was collected from December 2007 until October 2012 from five European Institutions in three countries. It consisted of 104 patients with histologically confirmed cervical cancer, according to FIGO´s classification disease stage I-IIB. Exclusion criteria were women with microscopic tumours that could not be identified on TVS or TRS and women that were not eligible for primary radical surgery. The clinical characteristics of the study cohort are presented in Table 4. The participating centers were the ultrasound unit at the Department of Obstetrics and Gynecology, Lund University Hospital, Lund and Karolinska University Hospital, Stockholm Sweden; the Gynecological Oncology Center, Department of Obstetrics and Gynecology, General University Hospital, Prague, Czech Republic; the Department of Oncology, Catholic University of the Sacred Heart, Rome and the Gynecological Oncology unit, Division of Gynecology, IOE, Milan, Italy.

Table 4. Characteristics of study participants, cohort I (n=104)

Characteristics	Number (%)
Clinical stage*	
IA2	7(8)
IB1	81 (78)
IB2	12 (12)
IIA	3 (3)
IIB	1 (1)
Histology	
Squamous	66 (60)
Adenocarcinoma	38 (37)
Adenosquamous	4 (3)
Treatment	
Hysterectomy	94/104 (90)
Trachelectomy	5/104 (5)
Lymph node dissection	104/104 (100)
Radiochemotherapy	5/104 (5)

*according to FIGO 2009

4.1.2 Study population II

The cohort from Karolinska University Hospital generating patients to all of the studies behind this thesis consisted of 93 women with biopsy verified invasive cervical cancer, referred to Karolinska University Hospital for treatment from July 2011 until September 2015. During this time 483 women were diagnosed with invasive cervical cancer in region Stockholm/Gotland according to Swedish Quality Registry of Gynecologic Cancer (32). All women in the region who are diagnosed with cervical cancer are referred to the institution for treatment and were therefore considered eligible. Women with all disease stages were considered for inclusion. Of the eligible 96 accepted participation. Three were excluded as final histology showed other disease than cervical cancer, (Figure 10). In Table 5, the characteristics of the study participants are presented. The median age was 45 years, majority 62% (58/93) had disease stage ≤ IB1 and 67% (62/93) had a squamous cell histology. Included in study II there was also a cohort of 21 healthy women who were recruited through an advert amongst hospital personnel at Karolinska University Hospital.

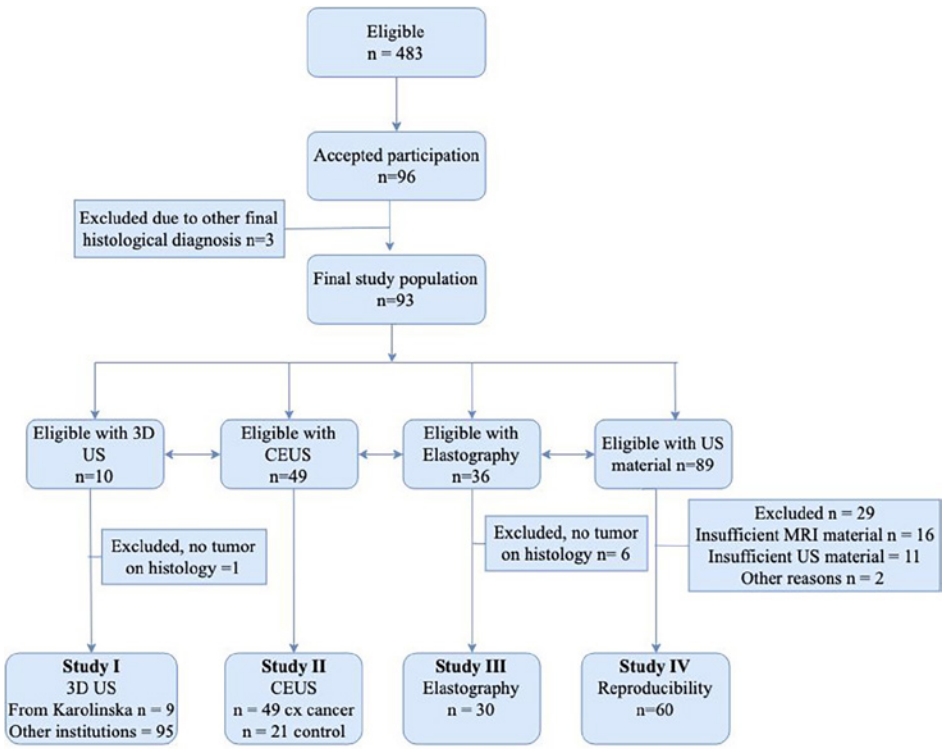


Figure 10. Flowchart of the study population II

Table 5. Characteristics of the study participants, cohort II (n=93)

Characteristics	Whole cohort (n=93)	Study II (n=49)	Study III (n=30)	Study IV (n=60)
*Age**	45 (24-85)	43 (24-76)	47 (26-71)	46 (24-85)
*Clinical stage***				
Earlys stages:	58 (62)	35 (71)	20 (67)	36 (60)
IA1	7	3	1	1
IA2	4	4	-	1
IB1	47	28	19	34
Advanced stages:	35 (38)	14 (29)	10 (33)	24 (40)
IB2	1	1	-	1
IIA	9	5	3	7
IIB	14	5	5	13
IIIB	8	2	1	2
IV	3	1	1	1
Histology				
Squamous	62 (67)	30 (61)	21 (70)	40 (67)
Adenocarcinoma	27 (29)	19 (39)	9 (30)	18 (30)
Adenosquamous	2 (2)	-		-
Other	2 (2)	-		2 (3)
Treatment				
Surgery	52 (56)	31(63.3)	18 (60)	31 (52)
Radiochemotherapy	41 (44)	18 (36.7)	12 (40)	29 (48)

median (range),according to FIGO 2009, the numbers are count (%)*

4.2 METHODS

The details of the methods applied for every study in this thesis is described in the papers attached. Presented here is an overview.

4.2.1 Study I

4.2.1.1 Study design and objectives:

Design: A prospective European multi-center diagnostic trial of 104 women with surgically resectable cervical cancer.

Objectives: To determine the clinical value of various objective 2D and 3D ultrasound parameters in comparison to subjective assessment to predict deep stromal and parametrial tumour invasion and lymph node involvement in women with cervical cancer scheduled for surgery.

4.2.1.2 Methods

The patients were staged according to FIGOs criteria for cervical cancer. The primary inclusion critera was surgically resectable disease and tumour seen with TVS or TRS. Most centers included only patients with disease stage IB1 or less, other centers accepted selected cases with more advanced tumours (stage IB2-IIB) for surgery (Table 4). The final histological diagnosis by reference pathologist at each center was based on tissue from radical surgery and lymphadenectomy, serving as gold standard.

4.2.1.3 Ultrasound examination

Examination and US systems

US examinations were performed with high-end systems by US experts (n=1-2) at each center prior to surgical treatment. All patients were examined with TVS or TRS in a lithotomy position with an empty bladder, scanning was done in sagittal and transverse planes. Still images with measurements, videos of the conventional grayscale, Power Doppler ultrasound examination, 3D grayscale and Power Doppler US were recorded at the same occasion for each patient. The results of the conventional grayscale US were assessed at the time of examination whereas the 3D volumes were retrospectively analyzed after completion of the study.

2D ultrasound and power Doppler

On the images from conventional 2D ultrasound the size of the tumour was measured, subjective assessment of the local extension of the disease, and presence of enlarged lymph nodes was evaluated. To begin with, the uterine cervix was centralized in the image. Secondly, the tumour was identified, often with help of power Doppler additionally to the grayscale. After identifying the tumour it was measured in three dimensions in millimeters; cervical fundal diameter, anteroposterior diameter and lateral diameter was measured where the tumour was largest. The tumour vascularization was subjectively classified during real time 2D power doppler ultrasound examination, using a color score: absent (=1), minimal (=2), moderate (=3), or high (=4), as introduced by the IOTA group for the assessment of ovarian mass vascularity (164) (Figure 6). The depth of stromal (>2/3) and presence of parametrial invasion (yes/no) was assessed at the level of the entry of the uterine arteries in both sagittal and transverse plane. If the tumour invaded through the pericervical fascia, parametrial involvement was suspected (120). Lymph node stations in the pelvis were scanned with abdominal US in addition to TVS / TRS.

3D ultrasound

After closure of the study the software program 4D view (GE) or QLAB (Philips) was used to calculate the volume of the tumour, vascularization index (VI), flow index (FI) and vascularization-flow index (VFI). The 3D volume calculation was done using 15 degrees rotational steps. Figure 11 is an example of a still image from the the 3D power Doppler view in a transverse plane in a patient with cervical cancer.

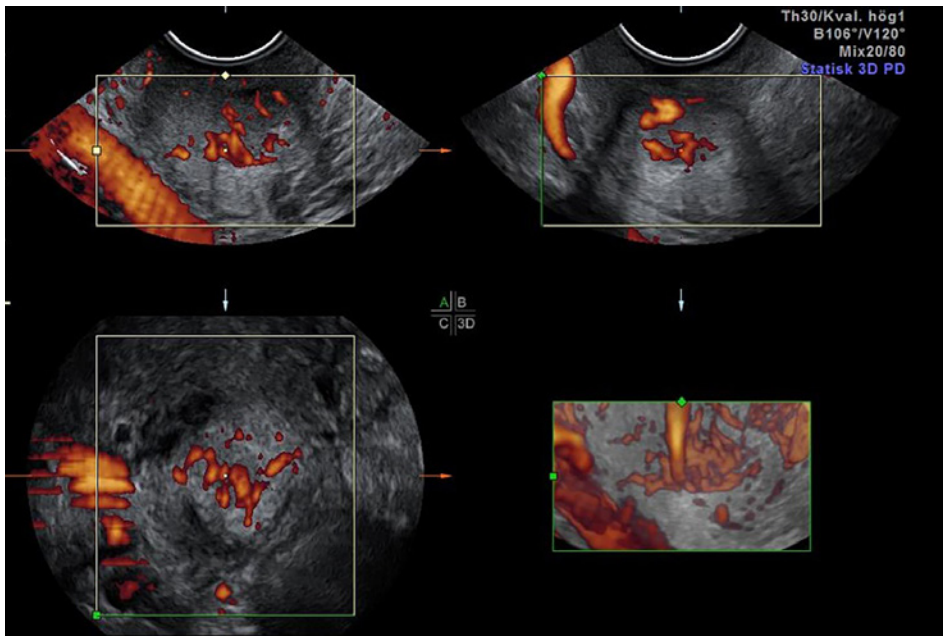


Figure 11. An image from a 3D PD volume, showing the vascularization of tumorous tissue from different angles.

Histological examination

Local reference pathologist at each center performed histological examination according to a predetermined protocol. The examination included assessment of tumour type, size, location, presence of > 2/3 of deep stromal and parametrial invasion, as well as assessment of lymph node metastasis.

4.2.2 Study II-IV

4.2.2.1 Study design and objectives

Study II

Design: A prospective single center pilot study including 49 patients with all stages of cervical cancer and 21 healthy controls.

Objectives: To describe contrast enhanced ultrasonography (CEUS) filling pattern

and semi-quantitative parameters in women with different stages of cervical cancer as compared to healthy controls. Furthermore, to assess the diagnostic accuracy and reproducibility of CEUS filling pattern as compared to conventional ultrasonography (US) for cervical tumour detection.

Study III

Design: A prospective single center pilot study including 30 patients with all stages of cervical cancer.

Objectives: The aim was to explore the features of Strain elastography (SE) in patients with different disease stages and to evaluate the accuracy of SE for size measurements and tumour delineation in comparison to conventional US alone using histology after surgery as gold standard.

Study IV

Design: A single center diagnostic cohort study of 4 different groups of raters reading US and MRI image material from 60 patients with all stages of cervical cancer

Objectives: To explore the inter-rater reproducibility of US and MRI raters with varying experience in a cohort of 60 patients with all stages of cervical cancer. Secondary aim was to evaluate accuracy in relation to experience and modality.

4.2.2.2 Methods (Study II-IV)

The patients were recruited at the first visit to Karolinska University Hospital after being diagnosed with invasive cervical cancer. As a routine at our institution, all the patients were clinically staged according to the FIGO's 2009 diagnostic criteria for cervical cancer (42). Patients with all clinical stages were considered for participation, and those accepting were was asked to participate in a conventional ultrasound examination and additional examinations with 3D, CEUS and SE. Not all patients accepted examination with all the different modalities, (Figure 10), leading to different number of patients included in to each study. However, every participant (n=93) was examined with conventional ultrasonography as a baseline examination by one of 2 specialists, one with over 20 years experience (EE) in this field and another (KP) with 4 years' experience on second-opinion ultrasonography. Cervical cancer US examination methodology and equipment has previously been described in our papers (165, 166) and chapter 4.2.1.3 in this thesis. All examinations were performed with TVS or TRS in combination with transabdominal ultrasound. Data was collected prospectively in case report form (CRF) on the day of ultrasound examination date, including information on

age and histology type according to biopsy. Subsequently clinical stage, date and outcome of surgery (type of surgical procedure and histological features) were filled out.

Magnetic resonance imaging (Used in Study IV)

According to routine at the institution all the patients included in study cohort II (n=93) were examined with MRI. At the time, imaging of the pelvis was performed with a phased array body coil using four different MR scanners. The images reviewed included high-resolution T2-weighted axial and sagittal images, oblique coronal images and oblique axial images (Figure 12) as well as T1-weighted axial images of the pelvis before, and after, administration of intravenous gadolinium-chelate contrast agent, either Gadopentetic acid (Magnevist 469 mg/mL, 0.2 mL/kg bodyweight, Bayer AB, Solna, Sweden) or Gadoteric acid (Dotarem 279.3 mg/mL, 0.2 mL/kg bodyweight, Gothia Medical AB, Billdal, Sweden). Diffusion weighted images were excluded as they were not available for the whole study cohort.

Histology (Study II-IV)

From cohort II (n=93), histology was available for surgically treated patients with early stage disease (n=52, 56%), (Table 5). According to routine at the department, a reference gynecology pathologist reviewed surgical specimens. Standard analysis for cervical cancer patients includes histological subtype, tumour dimension, depth of invasion to the cervical stromal, presence or absence of LVSI, parametrial invasion as well as analysis of the lymph nodes.

Contrast enhanced ultrasonography (Study II)

The CEUS examination of all the 70 participants, controls and patients, was performed using Philips IU22 US system with a 3– 9MHz transducer. Through a peripheral vein the injected bolus of 2,5 ml contrast agent Sonovue® (Bracco International B.V., Amsterdam, The Netherlands) was followed by 5 ml saline solution. For optimal orientation of the probe in relation to cervix, dual imaging was applied, splitting the screen for simultaneous CEUS and grayscale images. After injecting the contrast agent, a three-minute long video was recorded while holding the probe completely still. All the videos were analyzed at a separate occasion by two different examiners (EE and KP) several months after the closing of the study, to avoid bias. The readers were not blinded for case/control status of the image material.

The semi-quantitative analyses were done with Philips software package QLAB® (release 10, Philips Ultrasound, Bothell, WA, USA). The software uses modeling to

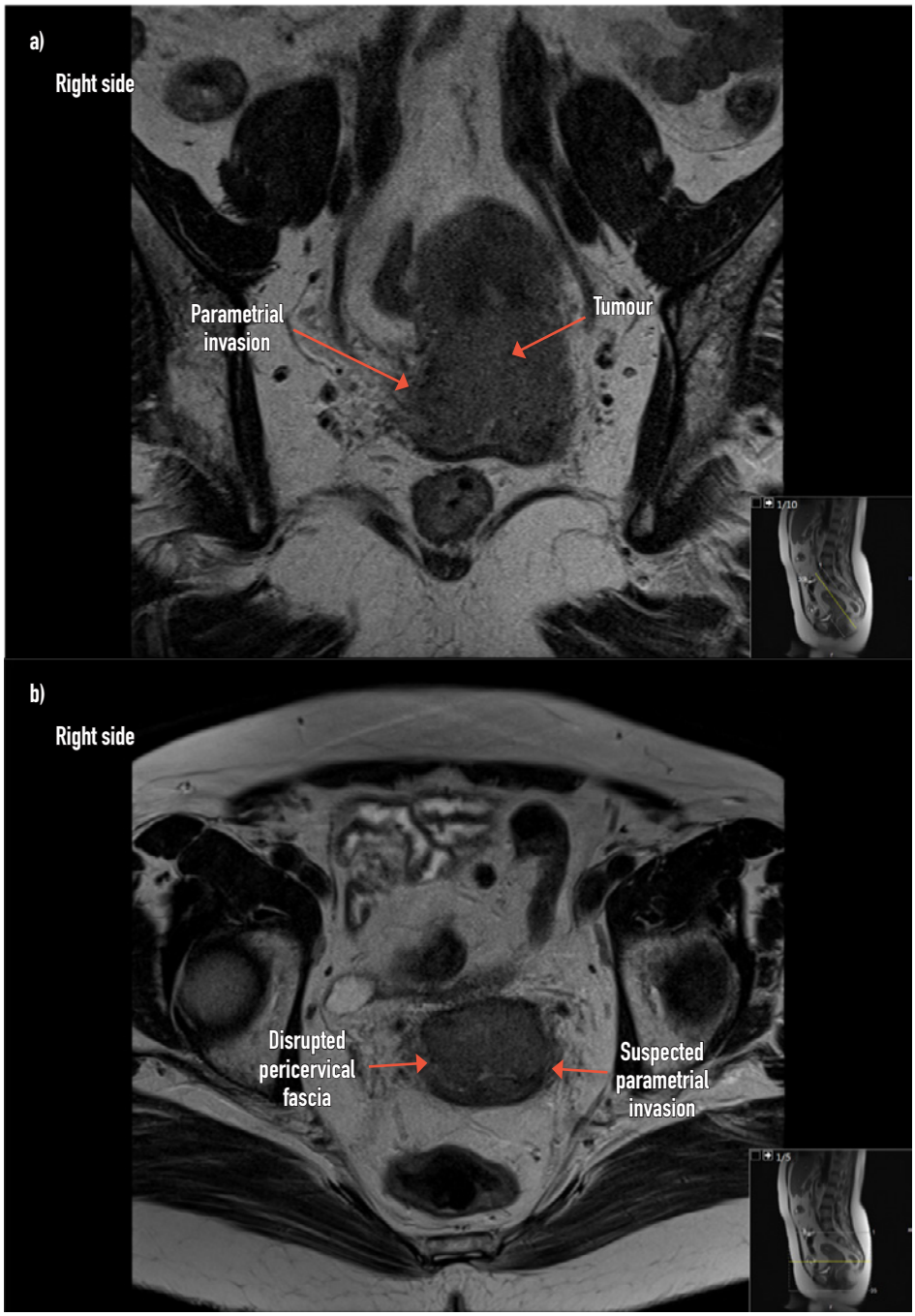


Figure 12. MRI for the same patient as featured in Figure 6, a woman with stage IIB cervical cancer. Figure **a** is from oblique coronary axis with arrow pointing at parametrial invasion on the patients right side, **b** is from transverse axial plane showing disrupted pericervical fascia to the right side and to the left also suspected minimal invasion to the parametrium. Images provided by: Susanne Fridsten.

draw a time-intensity curve (TIC) from a manually selected region of interest (ROI). A pre-fixed 5x5mm square was chosen to mark the 'max intensity area', while the ROI of the whole tumour/whole cervix was traced manually in different sizes, (Figure 13). Analyses were done on peak intensity of the contrast, time to peak, time to half wash out time and area under the curve (CEUAS-AUC) which reflects the intensity of the contrast during the specified time studied. Eleven patients and six controls were excluded from these analyses as their CEUS video had been saved in a format that could not be analyzed by the QLAB software.

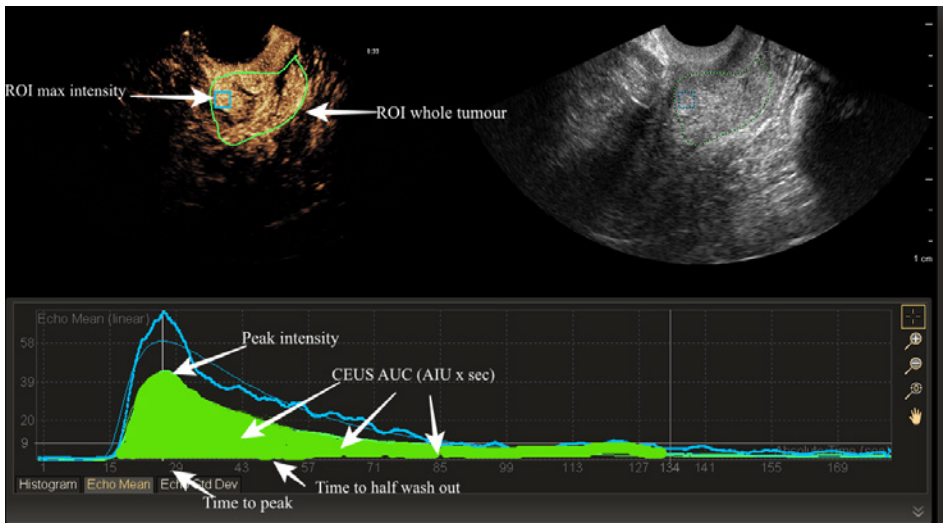


Figure 13. TIC curve for the semi-quantitative analyses of the contrast kinetics. Green circle is region of interest (ROI) around the whole tumour; blue box is ROI for max intensity of contrast. Furthest down the x-axis is the time in seconds; y-axis is the intensity of contrast. The whole green shaded area is the CEUS AUC for intensity of contrast under the examination time. AIU = arbitrary intensity units

For the qualitative analyses a simple subjective CEUS filling pattern classification system was invented where either a focal or a global filling (Figure 14) was seen in the uterine cervix. A wash out phenomenon, i.e. when a focal area looses the intensity prior to the surrounding was also observed.

Strain Elastography (Study III)

When the study was started, the plan was to use Voluson E8 ultrasound system with a 5-9 MHz transducer for the strain elastography examination. After having examined a couple of patients the image quality turned out very poor and fluctuating, why decision was made to change to Philips IU22 US system with a 3– 9MHz transducer. Due to this, the results presented in this thesis refer only to patients

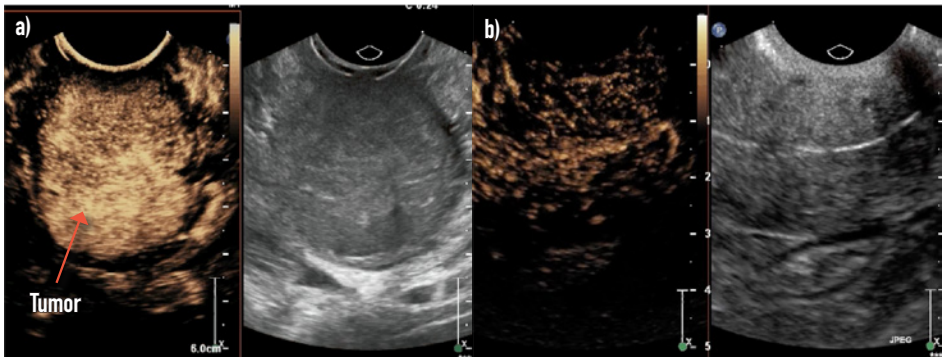


Figure 14. a) a focal filling pattern, arrow pointing at a tumour lesion, b) global pattern in a healthy cervical tissue.

examined with IU22. The SE examination was performed with TVS in conjunction to other US examinations.

Placing light compression on the cervix, SE images and videos 15 to 30 seconds long were recorded. The SE images were retrospectively analyzed off-line using Philips software package QLAB®, (release 10, Philips Ultrasound, Bothell, WA, USA) by the author of this thesis who had not been involved in the real time examination. The tumour borders were first delineated on the grayscale image, then the same is done on the SE image to determine if SE facilitated tumour detection and delineation. When tumour was identified on the SE images, size was measured in millimeters through cervical fundal diameter and anterioposterior diameter. Tumours were then classified by a five point elastography scoring system, using an accepted method that has been proposed for breast lesions by Itoh and colleagues 2006. The softest lesions are green receive a score 1, and the hardest lesions are blue with surrounding tissue blue as well score 5 (146), Figure 15 reveals examples of tumours with different elasticity scores.

Maximal strain ratio between the tumour lesion and the surrounding tissue was calculated in QLAB. Three patients with early stage disease were excluded from this analysis due to missing raw data. A pre-fixed 5 x 5 mm square was used, to mark the ROI and placed it at an area in the tumour and in the adjacent normal cervical stroma.

Raters and reviewing of images (Study IV)

Two groups of raters were recruited for both modalities; one with experienced raters and another group of raters without previous experience of assessing patients with cervical cancer. See Table 6 for overview of the experience for each group of raters. The ultrasound experts were European Gynecologists (n=6) working as second-opinion ultrasound experts. The group of less experienced ultrasound examin-

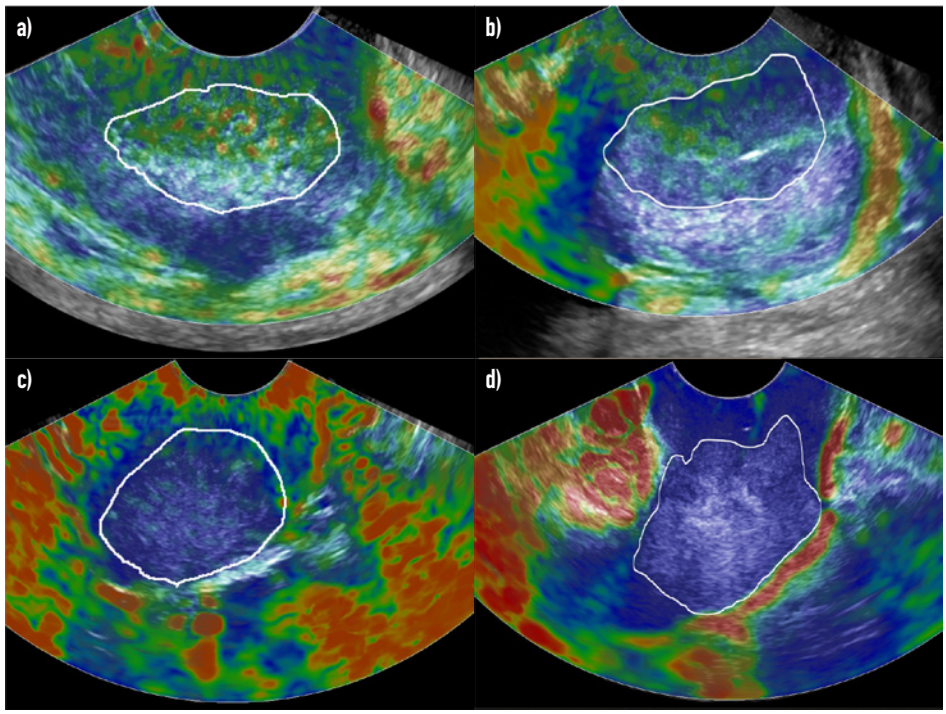


Figure 15. Elasticity score in different patients, white circle in the image is marking the tumour. a) Elasticity score 2, different shades of green/red in the tumour b) Elasticity score 3, mosaicism of blue and green c) Elasticity score 4, blue in the center but green in the periphery d) Elasticity score 5, tumour and surrounding tissue is blue

ers (n = 7), referred to as 'US specialists', consisted of Swedish subspecialized gynecologists practicing at different hospitals in Stockholm. For the MRI reading, nine raters from Karolinska University Hospitals participated, 5 certified Radiologists and 4 residents in radiology.

The US experts received a letter with a manual on how to execute the survey. An hour introduction lecture was held locally for the US specialists about ultrasound examination in patients with cervical cancer, giving examples on how to assess tumour visualization, 1/3 stromal- and parametrial invasion. Each of the US rater then received a flash hard drive and was asked to do off-line evaluation of the de-identified US images from 60 cases using their personal computers. MRI raters were introduced to basics of MRI reading for patients with cervical cancer during a two-hour lecture. The following two days they all reviewed the MRI images of the 60 cases, using a PACS workstation at Karolinska University Hospital under surveillance. All raters were informed that the cohort was a random mix including patients with all disease stages of cervical cancer.

Table 6. Experience of raters for both modalities.

Raters (n)	Years
US experts (6)	
Gynecologist	14,7 (5-24)
Second opinion US	10,5 (3-18)
Cervical cancer US	10,7 (3-18)
US specialists (7)	
Gynecologist	4,7 (2-8)
Second opinion US	1,3 (1-2)
Cervical cancer US	0
MRI experts (5)	
Radiologist	19,4 (10-30)
MRI	13,4 (4-25)
MRI pelvis	11,2 (1-25)
MRI residents (4)	
Resident radiology	4 (3-5)
MRI	0
MRI pelvis	0

(n) = number, years of experience are median (range)

For each case, there were three clinical questions with binary answers, yes or no; 1) Is there a visible tumour; 2) does the tumour infiltrate > 1/3 of the cervical stroma; 3) is there parametrial invasion? Raters answered all the questions parallel online through a personalized link to an online survey (Survey Monkey®), see US Survey link and MRI Survey link. Agreement and accuracy was calculated for each group of raters on all the questions except that accuracy for parametrial invasion could not be calculated as none of the surgically treated patient turned out to have disease in the parametrium on final histology.

4.3 STATISTICAL ANALYSIS

4.3.1 Study I-IV

A comparison was done of the mean (+/- SD) for normally distributed data and median (range) for non-parametric data. The chi-square test and Fisher´s exact test were used to compare categorical non-paired data as for color score, the Student´s t-test for normally distributed continuous data such as tumour diameter and the Mann-Whitney U-test for non-parametric data such as 3D and CEUS parameters. ROC curves with AUC and 95% Confidence Intervals were used to study diagnostic performance

in relation to sensitivity and specificity of different US and 3D variables in study I and for different CEUS variables in study II.

For study III Bland-Altman plot with 95% limits of agreement were used to compare size measurements from different modalities to histology. Comparing the index tests (SE and US) to the reference test (histology).

For study II and IV Cohen's Kappa (κ) was calculated for rater pairs and Fleiss' kappa was calculated for group of multiple raters. In all cases where Kappa was calculated, agreement was interpreted as follows; Poor for κ = 0 – 0.2, fair for κ 0.21-0.40, moderate for κ 0.41-0.60, good for κ 0.61-0.80 and very good for κ 0.81-1 according to Brennan and Silman (162). Sensitivity, specificity and accuracy for tumour seen, and stromal invasion > 1/3, were calculated for patients treated surgically, n = 31, using final histology as gold standard. Accuracy was calculated as a proportion of cases correctly classified divided by total case number.

For all the studies a p-value < 0.05 was considered significant. All the analysis were done using SPSS (Statistical Package for Social Sciences) software version 20-25 (IBM, Armonk, NY, USA) except the Bland-Altman plots in this thesis that are made in Graph Pad Prism (Version 6.0 for Windows, Graph Pad Software, La Jolla, CA, USA).

5 RESULTS

5.1 STUDY I

A total of 104 patients were included, 99 were operated with radical surgery including hysterectomy or trachelectomy. Five patients were operated with lymph node dissection only as radical surgery was aborted due to findings of positive lymph nodes during surgery. Stage ≤ IB1 was found in 88/104 (85%) while 16/104 (15%) were assigned stage ≥ IB2. Squamous cell carcinoma was verified in 66/104 (60%), adenocarcinoma in 38/104 (37%) and mixed adenosquamous type in 4/104 (3%).

Patients with deep stromal invasion and lymph node metastases had bigger tumours in maximal diameter as measured with 2D US as well as larger volume as measured with 3D US (p=0.05), table 7. Tumours with deep stromal invasion and lymph node metastases had more often high color score, no differences were found on 3D vascular indices.

Table 7. 2D and 3D US parameters in tumours with and without deep stromal invasion (n=99) and lymph node metastases (n=104)

	Deep stromal invasion			Lymph node metastases		
	No	**Yes**	**p-value***	**No**	**Yes**	**p-value***
2D						
Max diameter	21.5 (10.1)	36.2 (11.9)	< 0.001	29.8 (12.6)	35.5 (14.1)	0.05
Color score						
Low-moderate	19	13	<0.001	28	5	0.065
High	17	50		48	23	
3D						
Volume	3.0 (0.1-29.5)	16.4 (0.7-94.2)	< 0.001	9.5 (0.1-94.2)	15.7 (0.1-69.9)	0.03
VI	33.0 (7.6-74.7)	30.4 (2.5-68.5)	0.77	33.3 (2.5-74.6)	30.3 (7.4-61.3)	0.81
FI	38.8 (26.1-64.0)	38.6 (28.2-53.0)	0.45	39.8 (26.1-64.0)	38.0 (30.4-45.7)	0.52
VFI	13.2 (2.4-36.7)	11.6 (0.7-34.5)	0.61	13.2 (0.7-36.1)	12.3 (2.4-26.2)	0.76

Data are given as mean for diameter (+/-SD), number for color score and median (range) for 3D parameters.
*Student t-test was used for diameter, Chi-square for color score and Mann-Whitney test for 3D parameters

To predict deep stromal invasion subjective evaluation of images had higher sensitivity and specificity than color score 4, 91% and 97% vs.79% and 52%, respectively, Table 8. Maximal tumour diameter had AUC of 0.83, sensitivity 91% and specificity 61% at cut-off 20.5 mm, 3D volume had an AUC of 0.85, sensitivity 79% and specificity 83% at cut-off 9.1 mm3. Vascularity parameters VI, FI and VFI had no value to predict deep stromal invasion (Figure 16).

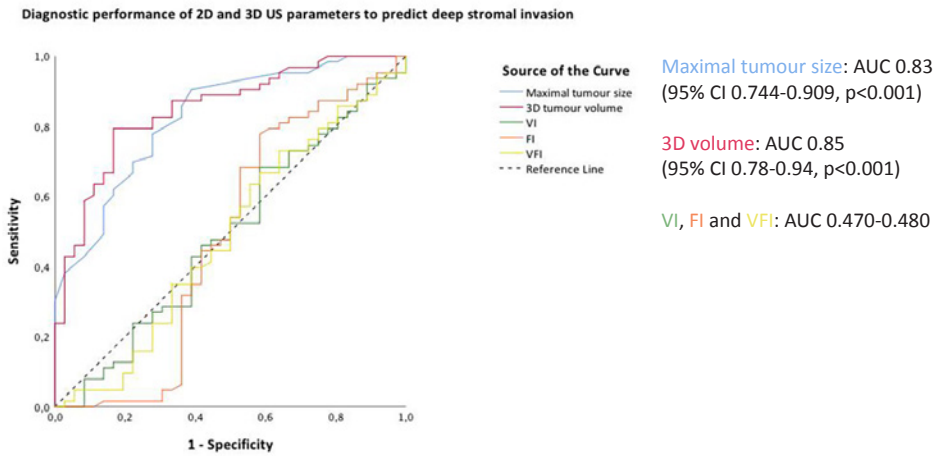


Figure 16. ROC curve for diagnostic performance of various 2D and 3D parameters to predict deep stromal invasion (n=99)

For detection of parametrial invasion subjective assessment had a sensitivity of 100% (7/7) and specificity 95% (87/92). As only 7/99 patients had confirmed parametrial invasion on surgical specimen, no analysis was possible to explore the predictive value of different US parameters in this subgroup.

For detection of lymph node metastases, subjective assessment had a low sensitivity and a high specificity, whereas prediction with color score 4 in the tumour tissue had a higher sensitivity than specificity (p<0.001), Table 8. All measurements from 2D or 3D parameters had poor diagnostic performance, see ROC curve (Figure 17).

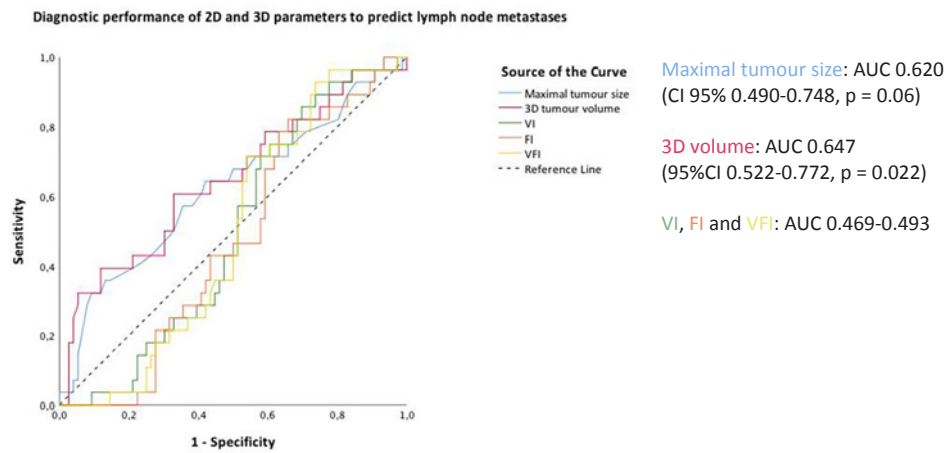


Figure 17. ROC curve for the performance of various 2D and 3D parameters to predict lymph node metastases (n=104)

Table 8. Accuracy of Subjective evaluation and 2D Power Doppler color score to predict deep stromal invasion (n=99) and lymph node metastases (n=104)

	Sensitivity	Specificity	PPV	NPV	p-value*
Deep stromal invasion					
Subjective evaluation	91 (57/63)	97 (35/36)	98 (57/58)	85 (35/41)	0.125
Color score 4	79 (50/63)	53 (19/36)	75 (50/67)	53 (19/32)	
Lymph node metastases					
Subjective evaluation	43 (12/28)	96 (73/76)	80 (12/15)	82(73/89)	<0.001
Color score 4	82 (23/28)	37 (28/76)	32 (23/71)	85 (28/33)	

All numbers in % (n/N) *McNemar Chi-square to test significance between subjective evaluation and high color score

5.2 STUDY II

The clinical characteristics of the patients are presented in Table 5. Median age of the patients was 43 (24-76) years. The clinical stages were the following, 35/49 (71%) had ≤ Stage IB1 and 14/49 (29%) stages ≥ IB2. Squamous cell carcinoma was diagnosed in 30/49 (61%) and adenocarcinoma in 19/49 (39%). There were no differences in the age (p =0.23) or menopause status (p=0.33) of the patients and controls.

Focal filling pattern was found in 3% (1/32) of the women with no tumour versus 89% (34/38) of women with histologically detectable tumour, Table 9. Four patients with a residual tumour at histology had global distribution of contrast enhancement. Three of those patients had clinical stage IB1 with very small tumours, the largest diameter measuring 6, 9 and 11 mm at histology, in total, eight patients had residual tumour size 1-20 mm. Further description of the CEUS pattern is presented in the paper from this study.

Table 9. CEUS filling pattern in the whole study cohort (n=70), light blue box represents subjects without tumour, green box represents subjects with tumour, all stages.

Filling pattern	Controls (n=21)	Stage ≤1B1, no tumour at histology (n=11)	Stage ≤ IB1, tumour at histology (n=24)*	Stage > 1B2 (n=14)
Global	21	10	3	1
Focal	0	1	21	13

*Including 4 patients who were not operated,

In patients with early stage disease (n=31 surgically treated), a focal filling pattern had a sensitivity of 80% (16/20), specificity 91% (10/11), PPV 94% and NPV 71% for tumour detection as compared to sensitivity 85% (17/20), specificity 73% (8/11),

PPV 85% and NPV 73% of subjective assessment with conventional US alone, the difference was not statistically significant (p=1.00 for sensitivity and p=0.62 for specificity respectively).

The inter-observer reproducibility in assessing the pattern of contrast distribution (focal versus global) was excellent (Kappa 0.91). The two examiners were in agreement in the case of 30/32 focal and 37/38 global patterns.

Table 10. Semi-quantitative analysis of Contrast-enhanced ultrasound (CEUS) time-intensity curves from the whole cervix in controls and tumour lesions in patients with cervical cancer (n=46)

Whole cervix or tumour	Controls (n = 15)		Cervical cancer with tumour (n = 31*)		p-value†
Time to peak (sec)	18.6	(14.0–39.1)	19.8	(9.3–51.1)	0.98
Peak intensity (AIU)	5.4	(0.6–17.9)	18.2	(0.6–57.0)	0.002
Area under the curve (AIU x s)	205	(40–1737)	314820	(45–4888786)	< 0.001
Half wash-out time (sec)	33.4	(16.1–112)	23.1	(9.3–112.7)	0.028

All numbers are presented as median (SD), † Mann Whitney t-test. *Seven patients without residual tumour at histology as well as eleven patients and six controls whose videos could not be analysed quantitatively were excluded.

The semi-quantitative analysis of contrast kinetics in healthy controls and patients with tumour is presented in Table 10. There was a statistically significant difference in the median peak intensity, area under the TIC curve (CEUS-AUC) and half wash-out time between healthy cervical tissue and tumour lesions. No difference was found between the groups on time to peak of the contrast.

CEUS-AUC had excellent accuracy and peak intensity had moderate accuracy to distinguish tumour from healthy tissue, (Figure 18).

Figure 18. ROC curve for the diagnostic performance of CEUS specific parameters for tumour detection, red line represent CEUS-AUC (Arbitrary Intensity Units x Seconds) of the contrast in whole tumour lesion, and blue line represents peak intensity.

5.3 STUDY III

There were 30 patients included in the final analysis, 6 were excluded because they had no residual tumour at final histology, (flow chart of study cohort). Of the 20 (66,7%) patients that had stage ≤ Ib1, 18 were surgically treated, (Table 5).

Tumour delineation improved in 40% (8/20) of early stage and 70% (7/10) of the advanced stages by adding SE to conventional US alone. Size agreement between SE and histology (n=18) was excellent with no bias found (mean size difference -0.11 mm, p= 0.657) by Bland Altman plot. The same was true for conventional US where the agreement was excellent as well, with no significant bias found (mean size difference 3.9 mm, p=0.488), (Figure 19).

An elasticity score of 4–5 was found in 45% (9/20) with early stage and 80% (8/10) with advanced disease (p = 0.068). The maximal strain-ratio was significantly (p < 0.009) lower in early stages (1.9; SD ± 0.8), compared to advanced stages (3,1; SD ±1.2).

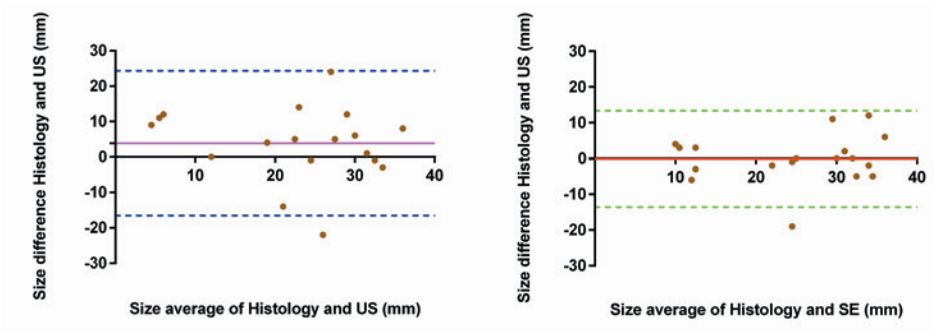


Figure 19. Bland Altman plot with 95% limits of agreement for tumour size measured with conventional US to the left where the pink line is the bias (mean difference). To the right strain elastography (SE) compared to histology after surgery (n=18), line for bias lies parallel to the reference line at zero.

5.4 STUDY IV

60 patients with all FIGO-stages were included for analysis by four different rater groups. The median time between US and surgery was 36 days (9-69), between MRI and surgery 42 days (4-71) and the median days between US and MRI was 5 days (0-45).

For all parameters studied; tumour seen, >1/3 deep stromal invasion and parametrial invasion both US expert and specialist groups had a moderate inter-rater agreement with Fleiss' kappa, (Table 11). The difference between the groups of experts versus specialists was not statistically significant for any of the questions

evaluated as seen by the overlapping CI. For MRI raters the agreement for the group of experts was good for all three questions with not statistical difference for Fleiss' kappa compared to MRI residents except for evaluation of tumour seen where residents had moderate agreement.

The sensitivity, specificity and accuracy for all the individual raters are presented in paper IV in this thesis. The range of accuracy for tumour visualization was as follows: US experts (68-74%), US specialists (61-77%), MRI experts (65-85%) and MRI residents (71-74%). For deep stromal invasion: US experts (58-74%), US specialists (48-74%), MRI experts (71-81%) and MRI residents (73-77%). As presented in Table 12 there was no statistically significant difference on sensitivity, specificity or accuracy between the groups with different experience for either modality.

Table 11. The inter-rater agreement for diagnostic evaluation of study cohort (n=60) with Fleiss' kappa for all rater groups.

Group of raters	Fleiss Kappa	95% CI	p-value*
Tumour seen yes/no			
US experts	0.460	0.395-0.525	<0.001
US specialists	0.462	0.407-0.518	<0.001
MRI experts	0.696	0.616-0.776	<0.001
MRI residents	0.513	0.409-0.616	<0.001
Deep stromal invasion yes/no			
US experts	0.445	0.380-0.511	<0.001
US specialists	0.525	0.470-0.581	<0.001
MRI experts	0.804	0.724-0.884	<0.001
MRI residents	0.709	0.606-0.812	<0.001
Parametrial invasion yes/no			
US experts	0.573	0.507-0.638	<0.001
US specialists	0.442	0.387-0.497	<0.001
MRI experts	0.691	0.611-0.771	<0.001
MRI residents	0.711	0.607-0.814	<0.001

p-value for testing wether Fleiss' Kappa > 0

Table 12. The diagnostic accuracy of all rater groups (n=31)

	Tumour seen					Deep stromal invasion				
Raters	**yes**	**no**	**Sensitivity**	**Specificity**	**Accuracy**	**yes**	**no**	**Sensitivity**	**Specificity**	**Accuracy**
US experts	117	31	85%	35%	72%	61	24	64%	74%	69%
	21	17				34	67			
US specialists	127	27	79%	52%	72%	53	15	48%	86%	66%
	34	29				58	91			
p-value*			0.181	0.101	0.731			0.101	0.295	0.445
MRI experts	78	6	68%	85%	72%	48	5	60%	93%	76%
	37	34				32	70			
MRI residents	67	10	73%	69%	72%	46	11	72%	84%	73%
	25	22				18	59			
p-value*			0.459	0.90	0.797			0.06	0.06	0.905

*Mann-Whitney

6 DISCUSSION

In this thesis three ultrasound modalities in addition to conventional US have been studied as well as inter-rater reproducibility of US and MRI raters with varying experience, in two cohorts of patients with cervical cancer. The main findings were:

Subjective assessment with conventional US had high accuracy to correctly diagnose patients with 2/3 deep stromal and parametrial invasion. Maximal tumour size measured with 2D US and tumour volume measured with 3D US was accurate to predict deep stromal invasion. All 3D PD vascular indices were useless for prediction of deep stromal invasion. To predict lymph node metastases, subjective evaluation was the best method with a reasonable PPV and NPV at > 80%, whereas a color score of 4 had a high sensitivity but low specificity, resulting in a PPV of 32% and NPV of 85%. Size measurement by 2D US or 3D parameters did not add any additional information in prediction of lymph node metastases.

Contrast kinetics were found different in cervical cancer tissue as compared to healthy cervical tissue with a higher peak intensity, higher CEUS-AUC as well as faster wash out time ($p<0.05$). The CEUS-AUC had very good performance to distinguish tumour tissue from healthy tissue. Focal contrast distribution was found in only 3% of the healthy controls and in cancer patients with no residual tumour after surgery versus in 89% of women with verified tumour. Tumour detection with the use of CEUS had in comparison to conventional ultrasound a higher specificity 91% vs. 73% at a similar sensitivity 85% vs. 80% although not statistically significant in this small series. The reproducibility for filling pattern was excellent between two readers ($\kappa = 0.91$).

Size agreement between SE and histology was excellent. An elasticity score of 4-5 was found in 45% (9/20) with early stage and 80% (8/10) with advanced disease and max strain ratio was significantly lower in early stages compared to advanced stages. Tumour delineation improved in 40% of cases with early stage disease, in contrast to 70% of the cases with advanced stages with SE compared to conventional US alone.

Reproducibility for US raters irrespective of experience was moderate for tumour visualization, > 1/3 deep stromal invasion and parametrial invasion. For MRI readers the inter-rater reliability was good irrespective of experience except for tumour visualization where residents had a moderate agreement. This difference in agreement was not reflected in accuracy as all rater groups had the same accuracy for tumour visualization and a not significant difference in evaluation of deep stromal invasion either, although the inter-rater range was wider for US raters.

6.1 THE RESULTS IN A CLINICAL CONTEXT

Study I and IV are more clinically applicable than study II and III which are pilot studies to explore a method that is previously not fully described or tested in patient settings. Taking into consideration the premises and limitation by a small study sample possible implications and clinical relevance will be discussed for all the studies.

6.1.1 Tumour detection and size

When the project started, an important issue was the unknown reference value for the kinetics of contrast in healthy cervical tissue. As a result of this, healthy women were included as controls in **study II**, which would now be considered one of the main strengths of the study. An assumption was made that the best dosage and the expected kinetics of the contrast would be similar to other organs with single arterial supply where recommended dosage is 2.4 ml as a bolus with intravenous injection (137, 167). A new classification system was invented with focal and global filling pattern. Although this system performed well with excellent inter-rater agreement and gave a higher specificity, it did not significantly increase the accuracy when compared to conventional US alone, possibly due to small sample size.

It was disappointing that in three out of eight small tumours < 20 mm no focal contrast filling was noted and the tumours were classified as having a global filling pattern. The findings suggest that assessment with the filling pattern might not work optimally in the smallest tumours even if this study is too small to draw definitive conclusion on this question.

Further results were the promising performance of CEUS with semi-quantification especially CEUS-AUC (enhancement/sec), to distinguish between tumour tissue and healthy tissue. These findings are supported by another study on 60 patients with all stages of cervical carcinoma where 50% more intensity of contrast was found in tumours compared to the reference tissue (myometrium of the uterus) even a faster time to peak was noted ($p < 0.001$). No differences in the contrast kinetics between different stages, tumour size or grade was found but a moderate correlation $rho=0.624$, $p<0.001$ between max contrast concentration and mean vascular density on histological specimens (168). Direct comparison of the quantitative measurements is not possible as different equipment was used with different units, even if the contrast agent was the same. It is interesting though that they used lower dose of contrast agent (1 ml vs. 2.5 ml) and still they had similar results. There is a certain risk for over-blooming of contrast related to higher dosage so according to these results our dose could be reduced. It is however doubtful that this would increase sensitivity of the filling

pattern for smaller tumours as they were classified with global filling with no signs of over-blooming pattern. Another important difference was that they used abdominal probes instead of the vaginal type applied in our and used myometrium as a reference standard. They consistently describe the same pattern as in **study II** with hyper-enhancement seen in the tumours in arterial phase and hypo-enhancement in the venous phase (wash out phenomena). Regardless of equipment or dosage of contrast there seems to be a window of 20-50 seconds after the injection of contrast agent where the contrast intensity peaks and allows evaluation the eventual tumour lesions before the effect has washed out again. Further validation of the contrast pattern of focal and global is motivated in a larger cohort as well as prospective trial to test different cut-off values for contrast intensity to explore if further advances can be made in the diagnostics of the smallest tumours.

Evaluation of tumour size with SE and grayscale showed good agreement with histology in **study III** with no bias detected for either modality. The narrower limits of agreement for size difference with SE than for conventional US indicates that SE might improve the precision of size measurement for small tumours. These findings do confirm those from two previous studies, one small but very meticulously done study on 18 patients with mainly stage IB1 and from Testa's study on 68 patients where no bias was found between histology and measurements with conventional US (105, 119). No studies have previously described the accuracy of SE for tumour measurements in this manner. To compare with previous findings on size measurements with MRI the largest study published 2007 on 150 patients with stage ≤ IB1 showed a mean size difference between MRI and histology -0.9 mm with 95% limits of agreement between -12.6 to +13 mm (91). There a better agreement in tumours > 10 mm was noted, where the mean difference was 0.3 mm and the limits of agreement were -7.5 to +7.9 mm. The limitations of that study were the low overall accuracy for tumour detection, and high number of misclassified patients (20/60) with residual tumour suspected on MRI where only inflammatory changes were seen on histology. Our preliminary results give the impression that the use of SE to measure size is accurate in early stage disease however not superior to conventional US solely or MRI.

The accuracy of MRI raters (sensitivity 68%, specificity 85%) on tumour detection in **study IV** are almost exactly the same as in the previously mentioned MRI study from 2007 as well as what Epstein and colleagues reported for MRI on 182 patients with early stage disease where sensitivity was 67% for tumour detection and specificity 89% with overall kappa = 0.52 between MRI and histology(1). On

the other hand, lower sensitivity 85% and specificity 37% for US experts was found when compared to Epstein's study where the US raters had 90% sensitivity, 97% specificity. Indicating that tumour assessment in live settings with US seems to have higher accuracy when compared to off-line readings of image material, and generally lower inter-rater reliability than for MRI in these settings.

6.1.2 Deep stromal invasion

What are the benefits of improving accuracy for detecting deep stromal invasion? The depth of stromal invasion has been linked to increased risk of recurrence and worse outcome in patients with early stage cervical cancer (78, 169, 170). In a large retrospective study aimed to find risk factors for parametrial invasion, data was prospectively collected for 842 cervical cancer patients with stage IA1-IB1 during an 18-year period 1984-2002. The incidence of parametrial invasion was 6% overall, on the other hand for patients with tumour < 2 cm, no lymph node metastases and depth of invasion ≤ 10 mm the rate of parametrial invasion was only 0.6% (171). The authors concluded that there might be a certain low risk group of patient that is suitable for less radical surgery. Several small case series, of patients conservatively treated, report very good oncological outcome for patients with stage ≤ IB1 with low risk histology subtypes, tumour size < 2 cm and varying grade of deep stromal invasion and LVSI (75, 172-174). No prospective randomized trials are published but the ongoing SHAPE trial randomizes patients with tumours < 2 cm, stage IA2 or IB1 with stromal invasion ≤ 10 mm on cone biopsy or stromal invasion 50% or less on MRI to radical surgery or simple hysterectomy together with pelvic lymph node dissection. The primary results are awaited next year. This is especially important for younger patients with microscopic disease where retained fertility is a major issue. With highly accurate diagnostic imaging where remaining disease or depth of invasion in the cervical stroma could be excluded, some patients could be counseled less radical surgery

According to the results in **study I** in addition to those previously presented from Testa and Epstein, Table 3 (1, 105) the subjective assessment is accurate and the best way to evaluate deep stromal invasion. Even if maximal tumour size with cut-off 20.5 mm had a high sensitivity to predict deep stromal invasion, a size that is described in many studies as the limit for a low risk disease (76, 171, 175), it is unlikely that any US parameters will ever perform better as a predictive factor than the highly accurate subjective assessment.

6.1.3 Parametrium

Evaluating the parametrium or pericervical tissue is one of the main criteria when selecting patients for either primary surgical treatment or RCT. Clinical evaluation has been reported to have low sensitivity but high specificity and a moderate inter-observer agreement for experienced clinicians for parametrial invasion (38, 41, 96). In **study I**, a high accuracy for evaluation of tumour in the parametrium was found with a negative predictive value of 100%. The high accuracy for conventional US evaluation of parametrial invasion **in study I** is in line with results from previously published studies (1, 104). Even MRI has previously been reported to have both high sensitivity and specificity for parametrial invasion as is discussed in the background, (Table 2) (96). With such high accuracy found for subjective assessment on conventional US in the settings of **study I**, any additional value of SE, CEUS or 3D is both unmotivated and impossible to study as the patients will be guided to primary RCT instead of surgery.

In **study IV**, a moderate agreement for both experienced and less experienced US raters was found for parametrial evaluation. The agreement for MRI raters was good irrespective of experience. For both modalities the agreement for less experienced raters was better than the reported agreement ($\kappa = 0.03$) for less experienced raters had with clinical examination in another study (41) as well as the reliability found between 4 raters for parametrial invasion ($\kappa = 0.11$) in the ACRIN/GOG study (156). One reason for the difference between the agreement of MRI raters in this study compared to the ACRIN/GOG study is the high proportion of patients with advanced disease, much smaller number of patients included and improved image quality in **study IV**. One explanation for the lower reliability found for the US raters is the lacking perception of the organs under examination, an important part of the life examination. As a conclusion, both MRI and US will increase the diagnostic accuracy for the evaluation of tumour in the parametrium when compared to clinical examination alone with generally higher agreement for MRI raters in off-line settings.

6.1.4 Lymph nodes

To date available diagnostic imaging modalities lack accuracy to detect metastases in the lymph nodes especially in low volume disease. One of the strengths of **study I** is the relatively high number of lymph node metastases in patients treated with surgery that made it possible with adequate sample size to evaluate the 3D US. Subjective assessment of lymph node metastases had a reasonable PPV and NPV (around 80%) in **study I**, however, a low sensitivity restricting the method. As low

sensitivity was expected, the aim was to study more specifically if 2D or 3D PD vascular indices would have some additional value to predict which patients would have lymph node metastases. Color score 4 was found in 23/28 patients with lymph node metastases with 2D PD and in 48/76 that did not have metastases, giving a very low PPV. So even if the tumours were generally well vascularized, irrespective of the findings of positive lymph nodes or not, the 3D vascularity indices turned out to be non informative for prediction more advanced disease. These findings are in agreement with two other studies showing no correlation between 3D PD indexes and lymph node metastases (126, 127). In these studies, the same US system was used and 4D view was used for analysis so the results are comparable. Our results are further confirmed by Alcazars findings on the uselessness of 3D-PD indices to predict recurrence after RCT in patients with locally advanced disease (130). Summarizing the published evidence, there is nothing to support the value of 3D-PD indices as a predictor for finding lymph node metastases or for monitoring therapeutic response in patients with cervical cancer.

It is interesting to put the results from **study I** in context with the results of **study II**. An abundant vascularity/high color score in almost all cervical tumours using Power Doppler in **study I** is in agreement with previous studies on cervical tumours (116, 176). As 3D PD vascularity indices were non-informative, another interesting aspect is the quantification of contrast kinetics as seen in **study II** as a surrogate for neovascularization. Zheng and colleagues found that the micro vessel density of small blood vessels in cervical tumours correlates to intensity of contrast (168). Higher micro vessel density in cervical cancer has been linked to worse survival for patient with node negative disease stage IB1 (177), moreover, the most recent addition to the therapy arsenal for patients with advanced and recurrent cervical cancer is an anti-angiogenic agent (178). This makes CEUS more interesting as a possible diagnostic method in the future for clinical applications such as examining lymph nodes, association to prognostic factors or for monitoring specific therapy response.
Metastases in the lymph nodes and parametrial invasion are poor prognostic factors and these findings should be included in the evaluation when patients are triaged for appropriate treatment. The main goal has been to avoid dual treatment with both surgery and radiation in patients with early stage disease as this increases morbidity without improving survival (80). The future role of predicting lymph node metastasis using imaging in patients with early stage disease is uncertain, as the question remains unanswered if radical lymph node dissection in patients with early stage cervical cancer is only diagnostic and not therapeutic. Some evidence

supports radical lymph node dissection as a therapeutic procedure. According to Höckel and colleagues, who have presented survival data (96% 5 years OS) superior to other studies for surgically treated patients with cervical cancer (stage ≤ IIB) without postoperative adjuvant treatment, the radical lymph node dissection is the most important aspect of the surgical treatment and not the radical hysterectomy. This is included in a surgical concept called total mesometrial resection (TMMR) where surgical dissection follows embryological development with different anatomical definition (179, 180). This approach is appealing especially in the light of recently published evidence regarding worse survival with MIS, still, Höckel´s results are yet to be reproduced. Until further research is published, SNB has to be considered the best available diagnostic complement to insufficient diagnostic imaging on the lymph nodes in patients with early stage cervical cancer.

6.1.5 Locally advanced stages

In **study III** tumours in patients with advanced stages were more easily demarcated, had higher strain ratio and a tendency for a higher elasticity score even if it was not statistically significant compared to early stages, probably due to a small patient cohort. The max strain ratio found in tumours both early (1.87) and advanced (3.06) was lower than in previously published studies where a strain ratio of 4.5 was suggested as a cut-off between benign and malignant cervical tissue (152, 181). These difference are explained by different study populations as patients with benign conditions such as cervical fibroma and dysplasia were included in the other studies, another reference tissue used for the calculation of strain ratio (parametrium) and last but not least different US equipment was applied both for examination and software for the analysis.

It is not obvious how the quantification of strain ratio can be applied in the diagnostic workup of cervical cancer patients for the time being. It should definitely not be used in the differential diagnosis between benign or malignant lesions as suggested by previously published studies, as tissue biopsy must be the gold standard to rule out cancer. It is not applicable either to figure out if patients have an advanced tumour or not, as more detailed morphological analysis on the dissemination is needed before treatment is started. The strain ratio could on the other hand be further tested to evaluate response to given RCT as has been suggested in another small study on patients with advanced stage disease (153). One study has reported higher accuracy for parametrial examination by adding SE to TVS as compared to TVS alone, however in that study both sensitivity and specificity of TVS alone were much worse than in previous larger multicenter study (182) and

compared to our findings in **study I**. As a result, no firm conclusion can be drawn on the role of SE in the evaluation of patients with cervical cancer for the time being neither from our study or other published studies on the subject.

6.1.6 Reproducibility

What is measured by inter-rater reproducibility and what affects the agreement between different raters? In **study IV**, two questions were interesting; the first one was if different modalities are comparable for the evaluation of patients with cervical carcinoma, and secondly if experience affects how well raters agree. Even if the results have been discussed in clinical context, the three important variables that can have affected the outcome of this study deserve more attention; the cohort of patients being examined, the raters doing the evaluation and the method used.

The prevalence of the condition being examined is important as rare conditions are likely to give lower agreement between raters (183). This would not be considered the case in our study, as it was a mixed cohort with all disease stages representative for our institution. The time between the US and MRI was only 5 days so the risk for differences in the disease stage are small.

There is certain heterogeneity within the different rater groups and the lower agreement for US raters could partly be a reflection of this. The most important is the different number of raters for each modality. The MRI raters were fewer, came from the same hospital and did the image readings in settings similar to the usual clinical ones. The US raters on the other hand were from different institutions in different countries, reading the images in settings unlike the clinical ones as unfortunately, reproducibility studies in live settings with multiple raters for US are not feasible.

Finally, the methods have intrinsic different characteristics and are expected to have different sensitivity and specificity as has been discussed previously. A bias is most likely introduced by using off-line settings for a method that is strongly dependent on the live examination settings as is the case for US. The results of equal agreement between experienced and less experienced raters on both modalities support this.

The main goal of calculating accuracy for the raters in the study was to put the inter-rater agreement in better context and to see if the agreement really mirrors the accuracy, furthermore, to examine if the accuracy in the off-line settings is comparable to live settings for the US. As previously discussed the accuracy was generally lower for US raters than seen in previous accuracy studies for ultrasound for both question. As a conclusion, evaluation of US images is most accurate in the

hands of the performing examiner who should evaluate the images in context to the live settings, as the method is less reliable and less accurate in off-line settings.

6.2 METHODOLOGICAL CONSIDERATIONS

One of the main challenges of this thesis was the collection and analysis of the CEUS and SE material. Even if research was done prior to starting the studies, unexpected problems were first revealed in real life settings. As previously described there were technical problems in the beginning when using SE leading to lost study material and as a result of this the final study population became smaller than initially planed. The same was true for the CEUS as the software used for the analysis can only read raw files a substantial part of the study material could not be included in the semi-quantitative analysis as the files had been recorded in the wrong format. Further challenge is holding the probe still for a long time during the CEUS examination. To diminish the risk of error due to movement of the probe in the semi-quantitative analysis, the patients were examined in only in one plane. This is a challenge for the examiner rather than the patient and a restriction of the method.

The sample sizes in **study II** and **III** are small and it creates a low statistical power and generalizability. When small cohorts are divided into subgroups a risk of statistical error and random findings/associations is created. As the cohorts in the thesis consisted of a majority of cases with early stage disease, no definitive conclusions can be drawn on the more advanced stages.

6.2.1 Selection bias

When patients are not recruited to the study randomly or consecutively or they do not represent the intended spectrum of target disease a selection bias is introduced. All the patients to study cohort II are recruited from Karolinska University Hospital at the time of diagnosis. During the 5 years of inclusion to our studies, approximately 20% of the patients diagnosed with cervical cancer in the region participated. The rate was fastest for inclusion between 2012 midyear until late 2014 but inclusion in to the study was extended to 2015 because of lacking material for the CEUS analysis. To avoid selection bias a systematic invitation to the study was included in the first visit to the Hospital. It is impossible to exclude that for some reasons some patients were not invited to participate as no registration was held on how many were screened for inclusion. The same is true for **study I**, where each center was responsible for recruiting patients, no registration is available for how many were screened. A certain spectrum bias can be found in the first study as more advanced stages are included for surgical treatment than would be accepted

here in Sweden and other Nordic countries. When comparing our study cohort II to the overall population of patients in Sweden with cervical cancer the age is similar as well as the proportion of patients with different histological subtypes and stages.

6.2.2 Information bias

Is a bias created when the results of the index test (US examinations) are interpreted with awareness of the results of the reference test. It can also cause information bias if the readers/raters get more or less information than would be available in clinical practice. There is risk for this type of bias in our studies because the raters of the CEUS examinations were not blinded to the control/patient status of the study participants. The raters were not blinded either by the clinical stage of patients in **study II** or **III** that could have influenced how patients were subjectively classified into global/focal pattern or if SE was subjectively evaluated as useful or not. In **study IV**, the raters had no information on the clinical stage, histological results or the primary results of the index test, as a result the accuracy could be lower than expected.

6.2.3 Verification bias

When a nonrandom set of patients do not go through reference test or when a subset of patients are verified or selected by a third reference test (MRI in this case). This is an actual problem in our settings. Even though the patients are clinically staged according to FIGO, the actual selection for surgical treatment is in many cases based up on the MRI findings. This has been verified in other studies (92, 157). Especially would tumour size, parametrial invasion or strongly suspected lymph node metastases direct the patients to primary RCT and no reference test results are then available for comparison to the index test. This creates a bias as the index test is then evaluated on a preselected group that consists of the cases classified by the MRI examination. This could lead to underestimation of the accuracy of the index test.

6.2.4 Classification bias

The time between US examination and operation in the patients with early stage disease can have created a classification bias. The risk that the disease has changed between the index test and reference test can not be eliminated as there was over one month between the two in study cohort II. That would potentially underestimate the ability of the index test to correctly classify the disease. By asking questions with binary answers like was the case in **study IV**, the diagnostic performance

of a rater is probably not truly revealed as the answers in clinical settings are rarely definite. This can underestimate or overestimate the true accuracy.

Another type of classification bias can occur when the reference test does not measure what it should measure. As some factors of the histology examination are subjectively evaluated such as the deep stromal invasion such bias is possible.

6.2.5 Validity

6.2.5.1 Internal validity

All the US modalities studied in this thesis are additional methods where conventional grayscale with Power Doppler is a baseline examination. The methods are strongly interwoven and the impact of the basic 2D US examination when subjectively evaluating the additional usefulness of 3D, CEUS or SE is inseparable. As a result of this, the true accuracy i.e. for filling pattern with CEUS is impossible to evaluate separately. As published in the paper from **study II** there were two raters doing the pattern recognition separately with an overall agreement of 91% that strengthens the validity of the classification. By doing both the pattern recognition and the quantitative measurement further strengthens the findings. Even though the calculations/quantifications from software programs such as 4D view and QLAB are estimates it is still an endeavor to get closer to true value of the method. Other concern is the histopathological evaluation that was not performed by a study specific pathologist. There is inevitable inter-observer variation imbedded in the test used as reference test that weakens the internal validity.

6.2.5.2 External validity

There are limitations to the external validity due to the technical issues described with CEUS and SE as well as the bias included in the quantification both by the software and by the examiner. There is a learning curve when these methods are applied and errors occur as a result of this. The methods need further improvement, especially the software for analysis and validation is essential with further prospective studies before clinical application is actual. Further limitation is the small number of patients for **study II** and **III** especially patients with advanced disease. For early stage disease the external validity is better as the number of patients is higher and the results are validated with histological results. For the 3D examination, the external validity is good as we have reproduced previous study results and the method as such is much more extensively studied and developed compared to CEUS and SE. The results are applicable to settings with same equipment used and the reproducibility for the rater groups applies to off-line settings.

The two cohorts are different. In **study I** there is a certain heterogeneity as more advanced stages were included from European centers. That led to high incidence of lymph node metastases, higher than expected in our settings at Karolinska University Hospital and other Nordic centers, this is a concern for the external validity as most centers at least in the Nordic countries do not surgically treat patients with tumour size > 4 cm or with suspect parametrial invasion. It can also affect the predictive value of a diagnostic method when the prevalence of a condition is increased or decreased. However, in this case it provided the chance to evaluate the sensitivity and specificity of the conventional 2D US in a relatively large prospective cohort both for the evaluation of deep stromal invasion, parametrial invasion and for the lymph nodes and therefore has to be considered as one of the strengths of the study.

7 CONCLUSION

Assessment of deep stromal and parametrial invasion with conventional US is accurate in live settings and is done best by subjective assessment.

Three-dimensional ultrasonography does not improve the evaluation of patients with early stage cervical cancer, neither 3D-PD indices nor volume measurements add any value to predict deep stromal invasion or lymph node metastases.

The addition of contrast-enhanced ultrasonography may increase the accuracy of tumour detection both by pattern recognition and by quantitative measures.

Strain Elastography has high accuracy for measuring tumour size in patients with early stage disease and appears to facilitate tumour outlining in patients with locally advanced disease compared to conventional US alone.

In off-line settings, inter-rater reliability for local assessment of cervical cancer patients by US images is moderate while it is moderate-good for MRI raters, where the experience of the raters does not affect the outcome for either modality.

As confirmed in this thesis, the conventional US is an accurate method for tumour assessment, where additional modalities must be outstanding to improve the overall diagnostic accuracy. Only marginal effect was measureable by adding CEUS and SE to the conventional US in the studies and as a result, no clinical application could be established. As a conclusion, current evidence does not support the routine use of 3D US, CEUS nor SE as an addition to conventional US for the workup of patients with cervical carcinoma.

8 FUTURE PERSPECTIVES

Further improvements of the SE and CEUS technique are desirable to facilitate the application in clinical settings. If the methods become more user friendly, further studies could be motivated with focus on the potential benefit of CEUS in patients with early stage disease. In a prospective validation study the cut-off values for CEUS-AUC suggested in our pilot study could be tested and the histological characteristics of tumours in relation to CEUS kinetics as well. Another possibility would be to study if CEUS is applicable for lymph node assessment in patients with normal sized lymph nodes as the distinct focus of contrast is noted in cancer lesions.

The future challenge will be to perform high quality research on a relatively uncommon disease, where the available diagnostic methods have already reach an acceptable level of accuracy for some of the most relevant clinical questions. How is it possible to come one step further, for instance to fine tune the diagnostics of the lymph nodes or find the microscopic cervical tumour with imaging? The solution is unlikely to be discovered with small, single center studies, and resources should instead be put into well-organized, multi-center studies with adequate sample size to possibly make progress towards better results in the management of this important patient group.

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